The evaluation of the potential of *Tenebrio molitor, Blatta lateralis, Blaptica dubia, Hermetia illucens* and *Naupheta cinerea* for human consumption

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Notes

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters, especially within the materials and methods section, is therefore unavoidable.

Summary

The proximate, chemical and microbial analysis of five insect species were evaluated to determine the nutritional significance of consuming insects. Black soldier fly *(Hermetia illucens)* larvae (BSFL), rated by the European Union as the insect species to have the biggest potential in food and feed, were selected for further analysis to determine its functional properties as an indication of its processing potential. The BSFL were further processed into a vienna-type sausage and compared to a traditional pork vienna sausage in terms of its nutritional composition and perceived texture analysis.

The five insect species are a good source of nutrients, where *Tenebrio molitor* (mealworm) was found to have the highest fat content (35.7 $g.100g^{-1}$), whilst *Blatta lateralis* (Turkistan roach) was found to have the highest protein values (101.5 $g.100g^{-1}$). The insects were considered a good source of energy (averaging 24.12 $MJ.kg^{-1}$), crude fibre (ranging from 8.7 $g.100g^{-1}$ to 19.1 $g.100g^{-}$) and minerals, specifically iron and zinc. The amino acid profile of each insect species compared favourably to the daily requirement for the average adult, with the exception of methionine, which is considered to be the limiting amino acid in all of the insects tested. Oleic acid was the most prominent fatty acid (FA) in all of the insects tested, with values ranging from 11.6 % in *H. illucens* to 46.2 % in *Blaptica dubia* (Dubia roach). Linoleic acid was the highest PUFA and ranged from 3.3 % in *H. illucens* to 13.9 % in *B. lateralis*. Alpha-linolenic acid, was found in low concentrations, with the exception of *B. lateralis* (0.9 % - 1.5 % of total FA).

In terms of microbial safety, *T. molitor* and *H. illucens* contained high total viable counts and unsafe levels of *Enterobacteriaceae.* Blanching reduced microbial levels to less than 10 $cfu.g^{-1}$ which was below the recommended amount. Blanching is recommended prior to consumption or processing. The aerobic endospore count was low on both *T. molitor* (< 10 $cfu.g^{-1}$) and *H. illucens* (< 100 $cfu.g^{-1}$) and Salmonella was not found on either insect species. There was a slight growth of *Listeria* species, which could pose as a potential risk.

BSFL were found to have some functional properties, however, the extent of the functionality of BSFL in a paste form was somewhat limited. BSFL had limited water (± 104 %) and lipid (± 105 %) absorption capacities, and formed a gel that was too weak to retain its shape under pressure. BSFL had a poor emulsifying activity and antioxidant activity. Blanching the BSFL reduced some of the functional properties, but had no effect on water and lipid absorption capacity. Blanching did have a positive effect on the colour retention of the BSFL by preventing enzymatic reactions.

BSFL could successfully be processed into vienna-type sausages, however, they were inferior to the pork sausage in terms of moisture, protein content, hardness, gumminess and springiness. From a food safety standpoint, the BSFL sausages are considered safe to eat at day 0 and after 14 days vacuum sealed in refrigerated conditions. Ultimately, BSFL does have the potential to act as a meat alternative in the meat industry.

Opsomming

Die chemiese en mikrobiese analise van vyf insek-spesies is beoordeel om die sinvolheid, in voedingswaarde, van die eet van insekte te bepaal. Die larwes van die swartsoldaatvlieg (*Hermetia illucens*) (beter bekend as black soldier fly larvae of BSFL), wat deur die Europese Unie as die insek-spesie met die grootste potensiaal as voedsel en voer gereken word, is gekies vir verdere analise om die funksionele eienskappe daarvan as 'n indikasie van die verwerkingspotensiaal daarvan te bepaal. Die swartsoldaatvlieg-larwes is verder verwerk tot 'n worsie soortgelyk aan 'n Weense worsie en vergelyk met tradisionele vark- Weense worsies ten opsigte van voedingsamestelling en waargenome tekstuuranalise.

Die vyf insek-spesies is 'n goeie bron van voedingstowwe. Van die vyf het *Tenebrio molitor* (meelwurm) die hoogste vetinhoud (35.7 g.100g^{-1}), terwyl gevind is dat *Blatta lateralis* (Turkestan-kakkerlak) die hoogste proteïenwaardes het (101.5 g.100g^{-1}). Die insekte is 'n goeie energiebron (met 'n gemiddelde 24.12 MJ.kg^{-1}), ruveselbron (wat strek van 8.7 g.100g^{-1} tot 19.1 g.100g^{-1}) en mineraalbron, veral van yster en sink. Die aminosuurprofiel van elke insek-spesie het goed vergelyk met die daaglikse voedingsvereiste van die gemiddelde volwassene, met die uitsondering van metionien, wat as die beperkende aminosuur beskou is in al die getoetsde insekte. Oleïensuur was die prominentste vetsuur (VS) in al die insekte wat getoets is, met waardes vanaf 11.6 % in *H. illucens* tot 46.2 % in *Blaptica dubia* (Dubia-kakkerlak). Linoleïensuur was die hoogste poli-onversadigde vetsuur en het gestrek vanaf 3.3 % in *H. illucens* tot 13.9 % in *B. lateralis*. Alpha-Linoleïensuur, is in lae konsentrasies gevind, met die uitsondering van *B. lateralis* (0.9 % - 1.5 % van totale VS).

Rakende mikrobiese veiligheid, het *T. molitor* en *H. illucens* hoë totale lewensvatbare tellings en onveilige vlakke van *Enterobacteriaceae* bevat. Blansjering het mikrobiese vlakke na minder as 10 cfu.g^{-1} verminder, wat onder die aanbevole hoeveelheid was, en word aanbeveel voor eet of verwerking. Die aërobiese endospoor-telling was laag in *T. molitor* (< 10 cfu.g^{-1}) én in *H. illucens* (< 100 cfu.g^{-1}) en salmonella is nie in een van dié twee insek-spesies gevind nie. Daar was 'n geringe aanwas van die *Listeria*-spesie, wat 'n potensiële risiko sou kon inhou.

Daar is bevind dat BSFL 'n paar funksionele eienskappe het, maar die omvang van die funksionaliteit van BSFL in 'n smeervorm was effens beperk. BSFL het beperkte water-absorpsiekapasiteit (± 104 %) en lipied-absorpsiekapasiteit (± 105 %) gehad, en 'n jel gevorm wat te swak was om sy vorm onder druk te behou. BSFL het 'n swak emulsifiseringsaktiwiteit en antioksidant-aktiwiteit gehad. Blansjering van BSFL het sommige van die funksionele eienskappe verminder, maar nie 'n uitwerking op water- en lipiedabsorpsiekapasiteit gehad

nie. Blansjering het wel ’n positiewe uitwerking op die kleurbehoud van die BSFL gehad deur ensiem reaksies te voorkom.

BSFL kon suksesvol verwerk word tot worsies soortgelyk aan Weense worsies, maar hulle was minderwaardig teenoor die varkwors in terme van vogtigheid, proteïeninhoud, hardheid, klewerigheid en veerkragtigheid. Uit ’n voedselveiligheid-oogpunt word die BSFL-worsies geag as veilig om te eet by dag 0, en na 14 dae vakuum-verseël in verkoelde toestande. Uiteindelik het BSFL wel die potensiaal om as ’n vleis-alternatief in die vleisbedryf te funksioneer.

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List of Abbreviations

BPW - Buffered peptone water

BSFL – Black soldier fly larvae

DRC – Democratic Republic of the Congo

EA - Emulsifying activity

ES – Emulsifying stability

GC – Gas chromatography

FAO - Food and Agricultural Organisation

LAC – Lipid absorption capacity

MUFA - Monounsaturated fatty acid

PSS - Physiological salt solution

PUFA - Polyunsaturated fatty acid

SFA - Saturated fatty acid

TPA – Texture profile analysis

UFA - Unsaturated fat acid

WAC – Water absorption capacity

WHO - World Health Organisation

Chapter 1

Introduction

Edward O. Wilson, a renowned American biologist, once said 'If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos' (Corbey & Lanjouw, 2013). This statement emphasizes just how dependent we as humans are on insects on a daily basis and just how vital they are to our existence. With the world's human population predicted to increase from 7.2 billion in 2013 to 9.6 billion people by 2050, there are the impending questions surrounding sustainable food production to meet this growing demand for food, and the topic of consuming insects has grown in popularity (Mitsuhashi, 2010; Premalatha *et al.,* 2011). The practise of eating insects, known as entomophagy, dates back to the dawn of human evolution (Sutton, 1995) and to this day is still widespread across the world. To date, a recorded estimate of 2037 different insect species are eaten globally (Jongema, 2015) and contribute to over 2 billion people's diet worldwide (van Huis *et al.,* 2013).

Using insects as food and feed is desirable due to their high feed conversion efficiency (Nakagaki & Defoliart, 1991; Premalatha *et al.,* 2011), fast growth rate, high fecundity (Mitsuhashi, 2010; Nakagaki & Defoliart, 1991) and the fact that select species can recycle agricultural waste matter (DeFoliart, 1975). The biggest appeal for insects as food is the good nutritional profile. Insect species have been found to have protein contents that are generally comparable to that of animal protein (Bukkens & Paoletti, 2005; Chakravorty *et al.,* 2014; DeFoliart, 1992), with good amino acid profiles, which can complement diets that are high in maize and wheat (Bukkens, 1997). Insects are also a good source of energy (Ghaly & Alkoaik, 2009), fatty acids (Chakravorty *et al.,* 2014; Tzompa-Sosa *et al.,* 2014; Womeni *et al.,* 2009) and minerals, with especially high iron and zinc contents (Bukkens & Paoletti, 2005; Hopley, 2016; Pretorius, 2011). It is suggested that the commercialisation of insects can greatly reduce the incidence of malnutrition in many poverty stricken areas within Africa, as well as, provide a sustainable protein alternative in Western culture (Banjo *et al.,* 2006b; Bukkens, 1997; Moreki *et al.,* 2012).

Western consumers are less apprehensive about consuming insects in a processed form (Hartmann *et al.,* 2015; Tan *et al.,* 2015), however, to incorporate insects into a product the functional properties of various insect species needs to be fully understood. To date there is very little information regarding the functionality of insect protein with the main focus being on the functional properties of insect flour (Assielou *et al.,* 2015; El Hassan *et al.,* 2008;

Omotoso, 2006; Osasona & Olaofe, 2010; Womeni *et al.,* 2012). Insect flour typically has high water and lipid absorption capacities (Assielou *et al.,* 2015; Omotoso, 2006; Osasona & Olaofe, 2010; Womeni *et al.,* 2012), good emulsifying activities and good gelling capabilities (Assielou *et al.,* 2015; Omotoso, 2006; Osasona & Olaofe, 2010).

Before fully advocating the consumption of different insect species, the safety of consuming insects needs to be established. High microbial loads have been found on various insect species, and it is suggested that blanching be employed as a pre-treatment to ensure microbial safety (Banjo *et al.,* 2006a; Klunder *et al.,* 2012). Another safety concern that needs to be taken into account is the potential allergen risks associated with insect consumption (Phillips & Burkholder, 1995; Srinroch *et al.,* 2015).

The objective of this study was to investigate the potential of insects as a food ingredient by looking at the nutritional profile and microbial safety of five insect species (*Tenebrio molitor* larvae, *Blatta lateralis*, *Blaptica dubia*, *Hermetia illucens* larvae and *Naupheta cinerea*). Currently black soldier fly (*Hermetia illucens)* larvae is one of the main insects considered to have the biggest potential to be used in food and feed according to the European Union (EFSA Scientific Committee, 2015). The ultimate use of BSFL in food depends on its processing potential, specifically as a meat alternative. Therefore an exploratory investigation into the functional properties of black soldier fly larvae was done in order to understand its processing potential. It was suggested that in order to introduce insects as a commercial food source into Western culture, insects should be incorporated into familiar meat products (Hartmann *et al.,* 2015; Schösler *et al.,* 2012; Tan *et al.,* 2015; Tan *et al.,* 2016). The black soldier fly larvae were then manufactured into a vienna-type sausage and compared to a traditional pork vienna sausage in terms of nutritional composition, microbial safety and instrumental texture profile.

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Chapter 2

Literature review

2.1 Introduction

With 9.6 billion people predicted to be on the planet by 2050, there are impending questions surrounding sustainable food production to meet the inevitable growing demand for food (Mitsuhashi, 2010; Premalatha *et al.,* 2011). As a result of the increase in population and urbanisation, there is a subsequent decrease in the availability of land for agricultural developments to keep up with the growing demand for feed and food (Mitsuhashi, 2010; Premalatha *et al.,* 2011). This has lead researchers and consumers alike to explore alternative resources aside from the traditional agricultural crops, with a substantial amount of focus being on insects as feed for animals (Pretorius, 2011; Bosch *et al.,* 2014; Hopley, 2016) and food for human consumption. It has been suggested on numerous occasions that a Western diet is particularly dependent on protein from animal origins in most of their dishes (Yen, 2009) and Western consumers are beginning to seek alternatives to animal protein in efforts to shift to a more sustainable diet (Schösler *et al.,* 2012). The consumption of insects, known as entomophagy, has sparked increasing interest amongst scientists and environmentalists as a potential solution to the inevitable global food security and sustainability issues humans will be facing in the coming years (van Huis *et al.,* 2013; Verbeke, 2015). Entomologists at Wageningen University in the Netherlands believe insects can contribute to solving global hunger and were quoted in saying that 'edible insects can feed the world' (Yates-Doerr, 2015). The use of insects as an alternative protein source for humans is appealing due to their high reproductive rate and feed conversion efficiency, as well as, their high nutritional content, with special attention being given to their desirable protein content. Additionally, insects are suggested to be more environmentally friendly as they can recycle waste matter and they use much less space and water (Aarnink *et al.,* 1995; Oonincx *et al.,* 2010). The commercial farming of edible insects has been suggested to prevent over-harvesting of wild insects and decrease malnutrition (Hardouin, 1995).

2.2 Role of entomophagy

Insects play an important role in almost every ecological niche; ranging from the pollination of plants, to decomposing and recycling waste matter (Katayama *et al.,* 2008; van Huis *et al.,* 2013). Insects have also provided humans with varied commercial products over the years; examples being honey from bees (Bradbear, 2009), silk from silkworms (Yong-Woo, 1999), carmine dye from female cochineal insects for food colouring (Chung *et al.,* 2001) and resilin

which is used in the medical field (Elvin *et al.,* 2005). Insects have also inspired many novel ideas and mechanisms in engineering, such as silk proteins inspiring strong, elastic biomaterials (Lewis, 1992) and termite hills inspiring efficient ventilation systems in buildings (Turner & Soar, 2008). An important commodity that is often overlooked is insects as a feed and food source. The consumption of insects dates back to the dawn of human evolution, sometime before the existence of *Homo sapiens (*Sutton, 1995*)*. The consumption of insects is even mentioned in various places in the bible: “he (John the Baptist) ate locusts and wild honey” (Mark, 1:6, New International Version, 1978) and “There are, however, some flying insects that walk on all fours that you may eat: those that have jointed legs for hopping on the ground” (Leviticus, 11:21, New International Version, 1978). Entomophagy is the word used to describe the process whereby humans eat insects, but there is a large portion of people who consume insects across the globe on a day-to-day basis (Gahukar, 2011; Rumpold & Schlüter, 2013b).

Despite the fact that Western culture frowns upon the consumption of insects, it has been reported that an estimated 2037 different insect species are eaten globally (Jongema, 2015) and form an integral part of over 2 billion people’s diet worldwide (van Huis *et al.,* 2013). In Thailand consuming insects is not associated with rural or poor communities; it is eaten by both the rich and poor due to its desired taste and palatability (Hanboonsong *et al.,* 2013). They are also no longer just sold by street vendors. Insects can now be purchased along with your groceries in your local supermarket, and as a result, the concept of farming insects has grown and become an accepted form of income (Hanboonsong *et al.,* 2013). In China, importing insects for consumption has become a lucrative business, with 800 t imported annually from surrounding countries, with an estimated net worth of $11 million US a year (Hanboonsong *et al.,* 2013). Figure 2.1 shows the most commonly eaten insects globally

according to their order as a percentage of the total insects eaten (Jongema, 2015).

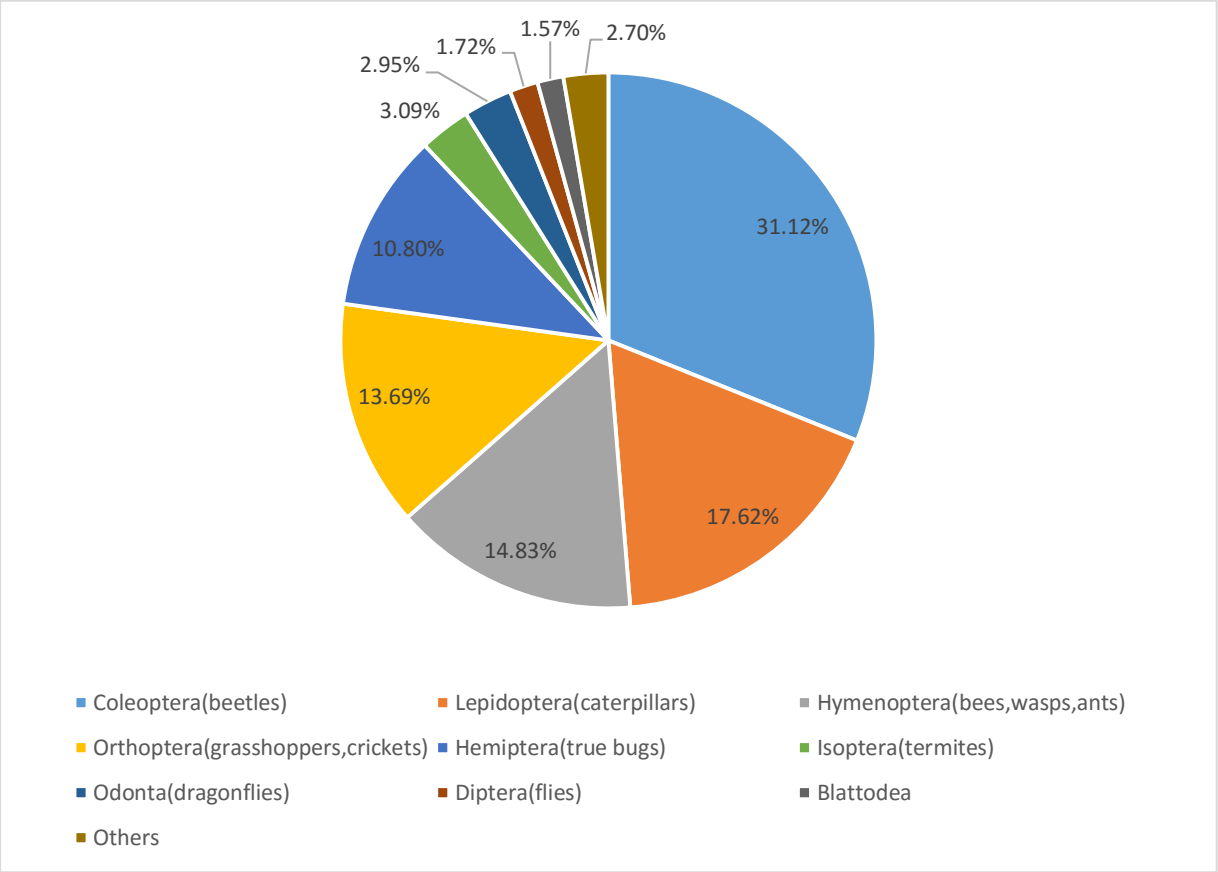

Figure 2.1 The most commonly consumed insects worldwide (adapted from Jongema 2015).

2.3 Entomophagy in Africa

A large percentage of the people who consume insects as a part of their diet are found in Africa. Within Africa, it is estimated that 96 different insect species are eaten, with the most common insects being from the orders Orthoptera (locusts and grasshoppers), and Lepidoptera (caterpillars) (Roulon-Doko, 1998). Different insects are eaten within the different regions in Africa, and this is attributed to the availability of insects in those regions, seasonality and cultural preferences. Due to the popularity of entomophagy in Africa, many research papers have been published regarding entomophagy in various African countries (Kozanayi & Frost, 2002; Banjo *et al.,* 2006b; Omotoso & Adedire, 2007; Madibela *et al.,* 2009; Womeni *et al.,* 2012; Riggi *et al.,* 2013). It has been reported that in Central Africa an estimated 50% of the total non-plant protein consumed is derived from insects, with an even higher percentage of 64% in the Democratic Republic of the Congo (DRC) (Raubenheimer & Rothman, 2013; Riggi *et al.,* 2013). The saying amongst the Yansi people in the DRC illustrates the importance of insects as food; “As food, caterpillars are regulars in the village, but meat is a stranger” (van Huis *et al.,* 2013).

The larvae of the palm weevil (*Rhynchophorus phoenicis*) and the rhinoceros beetle (*Oryctes monocerus*) are both delicacies in many Central African countries. These larvae are most commonly found in dead tree trunks and rotting vegetation and are typically harvested by the woman and children of the villages (Banjo *et al.,* 2006a; Moreki *et al.,* 2012). In Cameroon, where the palm weevils are a delicacy, they are only served in the company of good friends and family (DeFoliart, 1995). A dish containing palm weevils is highly regarded, and is most commonly prepared by cooking the larvae in an empty coconut with water and various condiments (DeFoliart, 1995). Termites are also an integral part of the diet in many Southern and Central African countries; namely Zimbabwe, Nigeria, DRC, Kenya and Uganda (Banjo *et al.,* 2006b; Ayieko *et al.,* 2010; Alamu *et al.,* 2013; Dube *et al.,* 2013). They are renowned for their high nutritional value, and are often fed to undernourished children in Uganda and Zambia (van Huis *et al.,* 2013). Typically termites are fried, smoked or sun dried, however, they are also sometimes steamed in banana leaves or ground and mixed with honey (Ogutu, 1986). Termite oil also has many applications, such as using it for frying, as well as, for hair and body treatments (Bergier, 1941; Dzerefos *et al.,* 2009). Aside from the termites being a source of nutrition, their nests also provide the ideal habitat for the growth of wild mushroom species, which can provide both sustenance and income to many families in countries like Nigeria (Parent & Thoen, 1977). The edible grasshoppers (*Ruspolia differns)*, an agricultural pest species, feeds many mouths in Eastern and Southern Africa. The grasshoppers appear in large numbers in the rainy seasons and consume the fields of sorghum and maize (van Huis *et al.,* 2013). The grasshoppers are harvested by hand and in Uganda 1 kg of grasshoppers is worth 40% more than 1 kg of beef (Agea *et al.,* 2008).

The Mopane caterpillar (*Imbresia belina*), endemic to Southern Africa, is the most popular caterpillar consumed on the continent, and is an important protein source for many of its people (Kozanayi & Frost, 2002; Moreki *et al.,* 2012). In some cases, the Mopane caterpillar can provide not only sustenance to families, but it can also provide them with a substantial income (Kozanayi & Frost, 2002; Stack *et al.,* 2003; Payne *et al.,* 2015). The Mopane caterpillar is highly sought after, and in many cases it generates more income than conventional agricultural farming (DeFoliart, 1992a; van Huis *et al.,* 2013). The Mopane caterpillar is popular even in countries as far north as the DRC, where it can be found in almost all of the food markets and is far more popular than any other caterpillar on the market (Kozanayi & Frost, 2002; Vantomme *et al.,* 2004). Mopane caterpillars are seasonal and found in abundance during Africa's summer months, namely March-May and November-January (Kozanayi & Frost, 2002; Moreki *et al.,* 2012). The most popular means of cooking the Mopane caterpillar is by first squeezing them by hand to remove the gut, boiling them in salt water, and then either sun drying them or roasting them over hot coals (Stack *et al.,* 2003). Another

commonly eaten insect in Southern Africa is the edible stink bug (*Encosternum delegorguei*). It is most commonly known for its foul odour and for being an agricultural pest, however, it is a delicacy and provides both sustenance and income to the Venda people in South Africa and the Norumedzo people of Zimbabwe (Faure, 1944; Teffo *et al.,* 2007). The stink bug has a chemical defence system that when secreted it discolours the harvester's hands and causes them to swell. When squirted directly into the eye of the harvester, the chemical causes a burning sensation and temporary blurry vision (Teffo *et al.,* 2007; Dzerefos *et al.,* 2013). Long term exposure to the defence chemical causes wart growth and nails to fall off of the nail beds (Dzerefos *et al.,* 2013). To ensure palatability, the chemical defence is removed either by physical removal of the glands, by heating or by a water method, whereby the stink bug secretes the chemicals into the water (Nonaka, 2009; Dzerefos *et al.,* 2013). These bugs are typically fried or dried and mixed in with porridge or eaten as a snack (Teffo *et al.,* 2007; Dzerefos *et al.,* 2013). As is the case of both the Mopane caterpillar and the stink bug, uncontrolled harvesting has led to the endangerment of these insect species (Sileshi & Kenis, 2010; van Huis *et al.,* 2013). Regulated conservation objectives need to be put in place that control harvesting (Gondo *et al.,* 2010). In the Norumedzo community, within the Bikita district of Zimbabwe, the importance of the stink bug to this community has led to designated stink bug protected areas, where tree felling is prohibited and no mechanical harvesting is allowed in order to protect and preserve both the crops and the stink bug (Makuku, 1998).

In Africa, food security is a reoccurring issue. There are many regions where protein is scarce, resulting in many people suffering from deficiencies (DeFoliart, 1999). This can be attributed mainly to the high cost of meat, the pressure on land space for livestock rearing, as well as, the distribution of meat focused mainly in the urban areas, leaving the rural and impoverished areas lacking good protein sources (Riggi *et al.,* 2013; Titilola *et al.,* 2015). Insects have always been a common protein source in many communities in Africa, and have been a large contributor to food security within these communities (van Huis *et al.,* 2013), however, due to the standard practise of using pesticides on crops, it is no longer safe to harvest and consume any insect that is found (Premalatha *et al.,* 2011; Temitope *et al.,* 2014). With the steady increase of protein deficiencies in Africa, a solution is the safe farming of insects, as it is a nutritious source of protein which is commonplace in African cultures (Bukkens, 1997; Riggi *et al.,* 2013). Insects have high protein contents, as well as, high iron, zinc and calcium (Finke, 2005). In Africa, iron deficiencies go hand in hand with protein deficiencies, and the farming of insects can provide a sustainable way of producing a safe protein and iron source (van Huis *et al.,* 2013). Additionally, insects contain many good fats and are a good energy source (Bukkens, 1997). Benin, one of the poorest countries in the world, is the ideal place for the development of insect farming, especially in the north where

the land will not accommodate conventional agricultural farming due to its poor soil quality and lack of grazing potential (Riggi *et al.,* 2013).

2.4 Benefits of utilising insects as food

With the world's human population increasing, there is a need for a constant food supply to meet the demands of the growing population (Mitsuhashi, 2010; Premalatha *et al.,* 2011). With the predicted increase in global population, more than half of the world's population growth in the next 100 years is predicated to occur in Africa, with populations in Nigeria predicted to surpass that of America, making it the second most populated country in the world (UN Press Release, 2013). Due to the increase in population and urbanisation, there will be a decrease in the availability of land for agricultural developments to keep up with the growing demand for food (Mitsuhashi, 2010; Premalatha *et al.,* 2011).

The cost of animal farming, as well as, the cost of food and feed security that goes along with animal farming has increased over the years (Moreki *et al.,* 2012). Currently 30 percent of the earth's total land space is utilised for agricultural purposes, and 70 percent of this agricultural land is used for livestock production (Premalatha *et al.,* 2011; van Huis *et al.,* 2013). Furthermore, 77 million t of animal and plant crops go towards animal feed on an annual basis, resulting in only 55 million t of plant and animal protein going towards human consumption (Premalatha *et al.,* 2011). The combination of increasing land pressure and the high cost to produce animal protein has resulted in increased protein deficiencies in both the middle and lower income classes (Oonincx *et al.,* 2010; Premalatha *et al.,* 2011). Developing countries are suffering the most, as they are facing shortages in animal protein resulting in high levels of malnutrition and growth deficiencies (Das *et al.,* 2009; Moreki *et al.,* 2012). Insects have been suggested for human consumption as an alternative to meat, to supplement the inevitable protein shortage.

The use of insects as food and feed is desirable as their feed conversion ratio is highly efficient due to the fact that insects are poikilothermic and do not use energy to regulate their body temperature (Nakagaki & Defoliart, 1991; Premalatha *et al.,* 2011) . Based on crickets, it has been estimated that between 80-83 % of the insects body is edible, whereas only 55 % of chickens and 40 % of pigs are edible (Nakagaki & Defoliart, 1991). Furthermore, insects produce large numbers of offspring and they have a fast growth rate, reaching maturity in a matter of days or weeks (Premalatha *et al.,* 2011; Riggi *et al.,* 2013). The house fly (*Musca domestica*) lays up to 500 eggs at a time, and with the removal of predators, the house fly can produce up to 2×250^{25} larvae per year (Mitsuhashi, 2010). On average one larvae weighs up to 25 mg, resulting in a potential 5×10^{46} million tons of larvae produced in one year alone (Mitsuhashi, 2010). Another example is the adult female cricket (*Acheta domesticus*), which

can produce between 1 200-1 500 eggs in a month, resulting in between 14 400-18 000 eggs in one year (Nakagaki & Defoliart, 1991). This demonstrates that when raised in the correct environment, insects can produce a substantial amount of edible protein (Nakagaki & Defoliart, 1991). It is therefore suggested that insects are an economically viable, environmentally friendly and energy efficient way of harvesting protein (Premalatha *et al.,* 2011).

Insects are estimated to produce little to no greenhouse gases (Oonincx *et al.,* 2010). In fact, according to Hackstein and Stumm (1994) methane is produced only by arthropods within the Diplopoda, Blattaria, Isoptera and Cetonidae taxa. The methane production is as a result of fermentation within the gut of these arthropods by methanogenic bacteria (Hackstein & Stumm, 1994; Wheeler *et al.,* 1996). As well as, having low greenhouse gas emissions, insects require very little water and land in order to rear them (van Huis, 2013; Titilola *et al.,* 2015). Some insect species also have the added benefit of being able to thrive on organic and agricultural waste, and can recycle and reduce organic animal and agricultural waste (DeFoliart, 1975).

Many of the insects that are consumed by humans are termed as agricultural pests, resulting in millions being spent on pesticides to eradicate them whilst at the same time taking food away from the families that rely on them for daily food and potential income (Ramos-Elorduy *et al.,* 1997; Premalatha *et al.,* 2011). In some communities, such as with the Norumedzo in Zimbabwe, harvesting by hand has become increasingly common practise as a means to harvest agricultural pests, as they are a source of nutrition and income for these communities (Makuku, 1998; Dzerefos *et al.,* 2009). The communities surrounding Lake Malawi are another example. Lake Malawi is home to countless numbers of lake fly larvae, and when fully mature they mate and swarm the area, consuming all the vegetation in their path. The inhabitants of this area welcome these pests, catching them by swinging woven baskets around in the air collecting enough flies to feed the entire community. These flies are then mashed up, made into patties and deep fried in oil, to produce a patty similar to that of a typical beef burger patty (Shaxson *et al.,* 1985). These nutritional and economic incentives can be used to create proper management of crops and agricultural pests in various communities in Africa, whilst harvesting the insects for food.

2.5 Farming insects

Due to the fact that entomophagy has mainly been practised in the more traditional and rural communities, the concept of farming insects on a large agricultural scale has been overlooked (van Huis *et al.,* 2013). This has resulted in the uncontrolled and unsustainable wild farming of insects which has now raised alarms for some insect species, specifically the Mopane caterpillar (I*mbrasia belina*), which could soon end up on the endangered species list (Sileshi

& Kenis, 2010; van Huis *et al.,* 2013). Fortunately, there is a strong drive towards the research and development of commercial insect farms, termed mini-livestock (Hardouin, 1995; van Huis *et al.,* 2013). Mini-livestock refers to small animal species, both vertebrate and invertebrate, that are bred for human food, animal feed or as a source of income (Hardouin, 1995; Titilola *et al.,* 2015). Mini-livestock are smaller than conventional livestock animals such as goats, cattle and poultry. Mini-livestock can contribute substantially to increasing food security in many African communities, as it can be set up in backyards and requires a small amount of input per unit output. It can therefore be used to supplement the diet and income of both rural and urban families (Hardouin, 1995; Titilola *et al.,* 2015). Insects are considered mini-livestock when they are utilised for human food, animal feed or as a source of income (Hardouin, 1995). Despite the need for insect farming, there are a few factors making it difficult to achieve. Certain insects are seasonal, making it difficult to rear and harvest them all year round. Additionally, mass rearing of select species is influenced greatly by their environment making it difficult to grow them outside of their natural habitat (Sileshi & Kenis, 2010). Optimising insect farming would create the potential for commercial insect farms for human food, be it eaten as is, or harvesting for further processing (DeFoliart, 1999; Sileshi & Kenis, 2010). Insect farming, has the potential to create an alternative, cheap, sustainable, environmentally friendly protein source that can be used to supplement conventional livestock farming (DeFoliart, 1999; Mitsuhashi, 2010; Premalatha *et al.,* 2011).

Silk worms are the most commercially farmed insects, and have been cultivated in China for over 5 000 years (DeFoliart, 1995; van Huis *et al.,* 2013). The domesticated silkworm is much larger than its wild counterpart, and is completely dependent on humans for its survival (van Huis *et al.,* 2013). Aside from the production of silk, the pupae of the silk moth became a common source of food for both humans and animals throughout China (DeFoliart, 1995). Silk moth pupae is a delicacy in many Asian countries, and is typically softened in water and cooked in an omelette, or added to stir fry as an alternative to meat (DeFoliart, 1995).

Controlled cricket farming was started in Thailand in 1998 with a recorded 22 340 farmers. In 2006 6 523 t of crickets were produced per year, and by 2011 it increased to over 7 500 t of crickets per year (Hanboonsong *et al.,* 2013). Hanboonsong and colleagues (2013) have described the common farming regimes that farmers have developed to efficiently farm crickets. The main problem facing insect farming is the inadequate research behind finding better and more efficient farming methods. Farmers are constantly facing new problems, but there is not much research in place to identify and solve the problems these farmers face (Hanboonsong *et al.,* 2013). More information is required regarding the various stages of insect production, as well as, post-harvest processing in order to further develop the edible insect trade in Thailand, and eventually across the world (Hanboonsong *et al.,* 2013).

Another means of harvesting insect protein has been explored by culturing insect cells in suspension in a bioreactor (Verkerk *et al.,* 2007). The bioreactor is a closed system with controlled conditions and has many advantages over conventional insect farming. The insect protein being produced is of a reproducible quality and has the potential for mass production, (Verkerk *et al.,* 2007). Furthermore, there is less risk of contamination and the composition and biomass of the cells can potentially be altered and controlled by using specific tissue types. In this way cells with the optimum requirements can be cultured (Mitsuhashi, 2002; Verkerk *et al.,* 2007). The advantage of culturing insect cells over animal cells is that insect cell cultures do not require equipment for carbon dioxide control, and they do not require strict temperature control, making it easier and more efficient to run (Mitsuhashi, 2010).

The concept of insect farming has also found its way into space travel. Currently one of the main challenges preventing extended space travel is the inadequate provision of food (Mitsuhashi, 2010). This has led to investigations into the utilisation of small scale insect farms in space agriculture (Mitsuhashi, 2010). There are two approaches to using insects in space agriculture. The first approach is to farm insects in combination with conventional small scale plant agriculture, whilst feeding the insects with the indigestible plant matter, and eating both the edible plant sections and the insects for protein (Katayama *et al.,* 2008; Premalatha *et al.,* 2011). The insects selected will depend on their nutritional content, their ability to recycle the waste that is produced in space, their reproduction rate and their ability to be reared in a small, confined space (Katayama *et al.,* 2008; Mitsuhashi, 2010). The second approach to insect protein production in space has been developed by Mitsuhashi (2002). This approach incorporates a continuous cell line process to harvest insect tissue in an artificial culture medium (Mitsuhashi, 2010). In order to culture insect cells under zero gravity, the culture vessels will need to be put under an artificial gravity created by a centrifugal force when rotating the culture vessels (Mitsuhashi, 2010). The disadvantage of using the continuous cell line process is that the organic waste on the space ship cannot be recycled, and will therefore not work as well in combination with small scale plant agriculture on the spaceship (Katayama *et al.,* 2008; Premalatha *et al.,* 2011). Due to the Astronauts long periods in space, with no available resources, space agriculture and the incorporation of insects in space agriculture will allow for longer and more in depth space missions to take place (Katayama *et al.,* 2008).

2.6 Nutritional aspects of insects

The consumption of insects can provide a range of nutritional benefits, which can in turn greatly reduce the incidence of malnutrition in many poverty stricken areas within Africa (Bukkens, 1997; Banjo *et al.,* 2006b; Moreki *et al.,* 2012). Table 2.1 shows the proximate composition of various edible insect species from across the world, highlighting their potential

as a nutritional food source. Only relevant data was included, and those that could be converted into the same units using the FAO INFOODS guidelines (FAO, 2012). The variation in nutrient content of insect species has been attributed to factors such as diet, environment and different stages in their lifecycle (Omotoso & Adedire, 2007; Xiaoming *et al.,* 2010; Oonincx, 2015).

It is widely known that insects contain a high protein content, with the order Lepidoptera containing protein contents ranging from 20 to 60 g per 100 g of dry weight. Orthoptera have protein contents ranging from 12.1 to 74 g per 100 g of dry weight and Coleoptera have protein contents ranging from 20 to 69 g per 100 g of dry weight (Table 2.1). These values indicate that the protein content of insects compare favourably with beef (40-75 g.100 g^{-1} DM) (Bukkens, 1997). The exo-skeleton of insects constitutes primarily of chitin, a long chain polymer, which contributes towards the insoluble fibre content of the insects (van Huis *et al.,* 2013). Reports have suggested that the chitin content may affect the protein results, and therefore the protein content of analysed insects may not be a good indication of the protein absorbed by the body (Bukkens, 1997). Analysis has shown that chitin only contributes to about 5-20 % of an insect's biomass depending on the species (Bukkens, 1997; Ramos-Elorduy *et al.,* 1997) and further studies done on *in-vitro* digestibility of insect protein showed that 77.9-98.9 % of insect protein is readily digested by human cells (Ramos-Elorduy de Conconi *et al.,* 1981; Assielou *et al.,* 2015).

In general, insects have high fat contents (Bukkens, 1997). Crude fat analysis of caterpillars show that they have fat contents ranging from 4.6 to 73.9 g.100g^{-1} DM depending on the species of the caterpillar (Table 2.1). Termite species commonly consumed in Kenya are particularly high in fat, with an average fat content of 46.59 g.100g^{-1} (Kinyuru *et al*., 2013). Fatty acid analysis shows that some insects contain a desirable amount of unsaturated fatty acids, with specific focus on both linoleic (C18:2n6c) (omega-6) (Table 2.3) and α-linolenic (omega-3) acid , both of which are vital in brain function (Bukkens, 1997; Tzompa-Sosa *et al.,* 2014; Zielińska *et al.,* 2015).

Table 2.1 Proximate composition of various edible insect species

Species	Common name	Country	% Moisture ($g.100g^{-1}$)	Energy ($MJ.kg^{-1}$) (DM)	%Crude protein ($g.100g^{-1}$) (DM)	Crude fat ($g.100g^{-1}$) (DM)	%Ash ($g.100g^{-1}$) (DM)	Reference
Lepidoptera								
Imbrasia ertli	Caterpillar	Angola, Congo	9.02	15.69	48.70	11.10	14.40	Moreki *et al*., 2012; Oliveira *et al.,* 1976
Utsa terpsichore	Caterpillar	Angola	9.24	15.52	44.10	8.60	11.80	Oliveira *et al.,* 1976
Gonimbrasia belina	Mopane caterpillar	Southern Arica	60.00-72.00	14.72-18.58	48.00-56.80	6.70-16.40	6.90-7.60	Bukkens, 1997; Ghaly, 2009; Payne *et al*., 2015
Hemijana variegata	Rothschild caterpillar	South Africa	-	21.08-23.11	51.41-53.84	18.93-19.33	5.23-5.53	Egan *et al*., 2014
Anaphe venata	Caterpillar	Nigeria	6.61-9.50	25.52	25.70-60.03	23.21-23.22	3.20-3.21	Ashiru, 1989; Banjo *et al*., 2006b
Galleria mellonella	Waxworm		-	-	41.75	51.40	3.30	Barker *et al*., 1998
Anaphe recticulata	Caterpillar	Nigeria	11.08	-	23.00	10.20	2.50	Banjo *et al*., 2006b
Chilecomadia moorei (larvae)	Tebo worms	Chile	60.20	12.46	38.94	73.86	2.01	Finke, 2013
Anaphe infracta	Silkworm	Nigeria	9.60	-	20.00	15.20	1.60	Banjo *et al*., 2006b
Anthoaera zambezina	Isoberlina paniculata	Zambia	60.90	14.52	56.70	9.20	12.50	Ghaly, 2009

Species	Common name	Country	% Moisture ($g.100g^{-1}$)	Energy ($MJ.kg^{-1}$) (DM)	%Crude protein ($g.100g^{-1}$) (DM)	%Total fat ($g.100g^{-1}$) (DM)	%Ash ($g.100g^{-1}$) (DM)	Reference
Hyalophora cecropia	Cecropia worm		-	-	54.70	10.20	5.90	Landry *et al.*, 1986
Callosamia promethea	Silkworm		-	-	49.40	10.00	6.90	Landry *et al.*, 1986
Manduca sexta	Carolina Sphinx worm		-	-	57.80	16.50	8.10	Landry *et al.*, 1986
Cirina forda	Emperor moth larvae	Nigeria	10.85	-	55.50	4.68	10.26	Omotoso, 2006
Spodoptera frugiperda	Fall armyworm	North America	-	-	57.20	11.30	11.20	Landry *et al.*, 1986
Spodoptera eridania	Southern armyworm	North America	-	-	54.40	14.90	6.90	Landry *et al.*, 1986
Pseudaletia unipuncta	Armyworm	North America	-	-	54.70	13.90	9.80	Landry *et al.*, 1986
Coleoptera								
Orcytes monoceros	Rhinoceros beetle	Nigeria	88.34	-	58.30	-	0.87	Banjo *et al.*, 2006b

Species	Common name	Country	% Moisture ($g.100g^{-1}$)	Energy ($MJ.kg^{-1}$) (DM)	%Crude protein ($g.100g^{-1}$) (DM)	%Total fat ($g.100g^{-1}$) (DM)	%Ash ($g.100g^{-1}$) (DM)	Reference
Rhynchophorus phoenicis fabr	Rhinoceros beetle	Zaire	13.70	27.66	24.30	55.00	1.00	Dufour, 1987
Rhynchophorus phoenicis	African palm weevil	Angola, Nigeria, Cameroon	10.10-10.75	23.51	20.34-28.42	31.40-41.73	2.39-5.53	Banjo *et al.*, 2006b; Womeni *et al.*, 2012
Tenebrio molitor	Mealworm		61.50-63.50	25.32	50.16 -68.87	27.20-31.17	3.70-5.70	Barker *et al.*, 1998; Ghaly & Alkoaik, 2009; Hopley, 2016; Yi *et al.*, 2013
Alphitobius diaperinus	Lesser mealworm beetle		64.50	-	58.02	25.94	-	Yi *et al.*, 2013
Zophobas morio	Superworm		59.90	27.95	43.13-51.62	38.21-40.80	2.68-3.50	Barker *et al.*, 1998; Hopley, 2016; Yi *et al.*, 2013
Analeptes trifasciata	Rhinoceros beetle	Nigeria	2.19	-	29.62	18.39	4.21	Banjo *et al.*, 2006b
Cirina forda	Caterpillars	Nigeria	31.56	-	20.2	14.20	1.50	Banjo *et al.*, 2006b
Orthoptera								
Locusta		Africa	57.10	18.24	47.50	22.90	-	Leung & Flores, 1961
Zonocerus variegatus	Grasshopper	Africa	62.70	7.11	26.80	3.80	1.20	Banjo *et al.*, 2006b; Bukkens, 1997
Brachtyrypes membanaceus	Cricket	Africa	76.00	4.90	13.70	5.30	2.10	Leung & Flores, 1961

Species	Common name	Country	% Moisture ($g.100g^{-1}$)	Energy ($MJ.kg^{-1}$) (DM)	%Crude protein ($g.100g^{-1}$) (DM)	%Total fat ($g.100g^{-1}$) (DM)	%Ash ($g.100g^{-1}$) (DM)	Reference
Acheta domesticus	House cricket		70.80	-	64.37-73.63	12.32-22.80	5.10	Barker *et al.*, 1998; Yi *et al.*, 2013
Cytancanthacris aeruginosus unicolor	Short horned grasshopper	Nigeria	9.20	-	12.10	3.50	2.10	Banjo *et al.*, 2006b
Chondacris rosea	Short horned grasshopper	India	43.62		68.88	7.88	4.16	Chakravorty *et al.*, 2014
Brachytrupes orientalis	Mole cricket	India	72.29		65.74	6.32	4.33	Chakravorty *et al.*, 2014
Hymenoptera								
Carebara sp.	Ants	Africa	60.00	-	3.00	9.50	/	Leung & Flores, 1961
Apis mellifera	Honeybee	Nigeria	8.70	-	21.00	12.30	2.20	Banjo *et al.*, 2006b
Isoptera								
Termese sp.	Termite	Kenya	40.00	17.32	28.80	32.30	-	Leung & Flores, 1961
Macrotermis bellicous	Termite	Nigeria	6.00-9.40	-	20.40-34.80	28.20-46.10	2.90-10.20	Banjo *et al.*, 2006b; Bukkens, 1997
Macrotermes notalensis	Termite	Nigeria	10.50	-	22.10	22.50	1.90	Banjo *et al.*, 2006b
Macrotermes nigeriensis	Termite	Nigeria	10.78	-	20.94	34.23	7.60	Igwe *et al.*, 2012
Termese sp.	Termite	Africa	44.50	27.45	35.70	54.30	4.80	Murphy *et al.*, 1991

Species	Common name	Country	% Moisture (g.100g^{-1})	Energy (MJ.kg^{-1}) (DM)	%Crude protein (g.100g^{-1}) (DM)	%Total fat (g.100g^{-1}) (DM)	%Ash (g.100g^{-1}) (DM)	Reference
Blattodea								
Blatta lateralis (nymphs)	Turkistan roach		69.10	6.70	61.48	32.36	3.88	Finke, 2013
Blatta lateralis (adult)	Turkistan roach		-	25.68	45.94	38.33	3.36	Hopley, 2016
Naupheta cinerea	Lobster roach		-	23.30	60.34	24.76	4.97	Hopley, 2016
Gromphadorhina portentosa	Madagascar hissing cockroach	Madagascar	-	23.43	55.28	24.46	4.56	Hopley, 2016
Periplaneta americana	Palmetto roach		-	26.09	49.03	37.27	3.42	Hopley, 2016
Oxyhaloa deusta	Cape red roach		-	24.99	52.59	28.85	3.78	Hopley, 2016
Diptera								
Hermetia illucens	Black soldier fly larvae		61.20	8.34	45.10	36.08	9.02	Finke, 2013
Musca domestica (adult)	House fly		74.80	3.84	78.17	7.50	6.74	Finke, 2013
Musca domestica (larvae)	House fly		-	20.10	60.38-63.10	9.30-15.5	5.30-11.90	Calvert *et al.,* 1969; Pretorius, 2011; Teotia & Miller, 1974

Species	Common name	Country	% Moisture (g.100g^{-1})	Energy (MJ.kg^{-1}) (DM)	%Crude protein (g.100g^{-1}) (DM)	%Total fat (g.100g^{-1}) (DM)	%Ash (g.100g^{-1}) (DM)	Reference
Drosophila melanogaster	Fruit fly		-	-	56.25	17.90	5.20	Barker *et al*., 1998
Hemiptera								
Encosternum delegorguei	Stink bug	Southern Africa	-	26.00	35.20	50.50	1.70	Teffo *et al*., 2007

The amino acid composition of most edible insect species corresponds well to the reference standards set out by the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) (Bukkens, 1997). Analysis of the amino acid profile, especially the essential amino acids, gives a good indication of the quality of insect protein (Bukkens, 1997). The amino acid content differs from species to species (Table 2.2) and this can be attributed to variations within the diet of the insects, as well as, climate and habitat (Bukkens, 1997; Verkerk *et al.,* 2007). In 2007 the WHO released a report containing the daily requirements of essential amino acids for the average healthy adult, as well as, infants and children (Joint WHO/FAO/UNU Expert Consultation, 2007). Table 2.2 below shows the WHO's recommended daily requirements for the essential amino acids for both adults and children. Infants require more of each amino acid than that of adults, and as they grow their daily requirement decreases until they reach adulthood. This emphasises the importance of amino acids in growth and development (Joint WHO/FAO/UNU Expert Consultation, 2007).

The amino acid composition of the mealworm (*Tenebrio molitor*) has values higher than that recommended by the WHO for both adults and children (Table 2.2), and can therefore be used to fulfil the daily requirements for amino acid consumption during growth and at full maturity (DeFoliart, 1995; Joint WHO/FAO/UNU Expert Consultation, 2007). This would be ideal in the poor communities in Africa, where they lack many of the essential amino acids (Bukkens 1997). In many African countries, especially the impoverished areas, maize is the staple food source. Both lysine and tryptophan are found to be limiting amino acids in maize. Limiting amino acids are essential amino acids found in low concentrations in the protein, resulting in the protein being of a low quality. This has resulted in a deficiency of both lysine and tryptophan in the African population (Bukkens, 1997; Joint WHO/FAO/UNU Expert Consultation, 2007). From Table 2.2 it can be seen that some insects, such as termites with their high lysine and tryptophan content would supplement a maize diet, which could in turn decrease the occurrence of lysine and tryptophan deficiencies (Bukkens, 1997).

Minerals are an integral part of the human diet, with great emphasis being placed on iron, zinc and calcium intake (FAO & WHO, 2005). Iron and zinc deficiencies are common nutritional disorders, particularly in developing countries (FAO & WHO, 2005; World Health Organization, 2001). Insects are high in both iron and zinc (Table2.4) and it is suggested that insects could be used to supplement the iron and zinc intake in malnourished communities (DeFoliart, 1992a; Bukkens & Paoletti, 2005; Banjo *et al.,* 2006b; Chakravorty *et al.,* 2014).

Table 2.2 Showing the essential amino acid profile of various insects (mg amino acid.g protein^{-1}) and the daily requirements of essential amino acids for both adults and children

Species	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Reference
Adult requirement (mg.g^{-1} protein per day)	30.00	59.00	45.00	16.00	6.00		38.00	23.00	6.00	39.00		15.00	Joint WHO/FAO/UNU Expert Consultation, 2007
6 months old requirement (mg.g^{-1} protein per day)	32.00	66.00	57.00		28.00		52.00	31.00	8.50	43.00		20.00	Joint WHO/FAO/UNU Expert Consultation, 2007
1-2 years old requirement (mg.g^{-1} protein per day)	31.00	63.00	52.00		26.00		46.00	27.00	7.40	42.00		18.00	Joint WHO/FAO/UNU Expert Consultation, 2007
3-10 years old requirement (mg.g^{-1} protein per day)	31.00	61.00	48.00		24.00		41.00	25.00	6.60	40.00		16.00	Joint WHO/FAO/UNU Expert Consultation, 2007
11-14 years old requirement (mg.g^{-1} protein per day)	30.00	60.00	48.00		23.00		41.00	25.00	6.50	40.00		16.00	Joint WHO/FAO/UNU Expert Consultation, 2007
15-18 years old requirement (mg.g^{-1} protein per day)	30.00	60.00	47.00		23.00		40.00	24.00	6.30	40.00		16.00	Joint WHO/FAO/UNU Expert Consultation, 2007
Lepidoptera													
Nudaurelia oyemensis	25.60	82.70	79.90	23.50	19.70	58.60	75.70	44.50	16.00	96.00	63.50	18.10	Bukkens, 1997
Utsa terpsichhore	108.70	91.30	91.00	11.30	12.90	55.90	33.00	50.80	6.60	75.80	-	-	Oliveira *et al.,* 1976
Imbrasia truncata	24.20	73.10	78.90	22.20	16.50	62.20	76.50	46.90	16.50	102.00	55.50	17.40	Bukkens, 1997

Species	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Reference
Imbrasia epimethea	28.60	81.00	74.20	22.40	18.70	65.00	75.00	48.00	16.00	102.00	66.20	19.70	Bukkens, 1997
Imbrasia ertli	36.00	36.70	39.30	15.80	13.40	17.40	13.20	40.50	8.10	41.90	-	-	Oliveira *et al.,* 1976
Aegiale hesperiaris	49.00	52.00	36.00	10.00	-	37.00	42.00	33.00	9.00	47.00	30.00	16.00	Ramos-Elorduy, 1987
Aegiale hesperiaris	49.00	52.00	36.00	10.00	-	37.00	42.00	33.00	9.00	47.00	30.00	16.00	Ramos-Elorduy, 1987
Anaphe venata	57.00	83.00	75.00	46.00	14.00	51.00	54.00	54.00	9.00	56.00	68.00	25.00	Ashiru, 1989
Hyalophora cecropia	33.00	51.00	44.00	22.00		140.00		41.00	-	45.00	53.00	27.00	Landry *et al.*, 1986
Callosamia promethea	29.00	44.00	43.00	15.00		114.00		39.00	-	41.00	42.00	29.00	Landry *et al.*, 1986
Manduca sexta	37.00	62.00	81.00	26.00		93.00		36.00	-	56.00	42.00	41.00	Landry *et al.*, 1986
Spodoptera frugiperda	38.00	77.00	77.00	31.00		128.00		51.00	-	52.00	67.00	38.00	Landry *et al.*, 1986
Pseudaletia unipuncta	38.00	64.00	180.00	36.00		93.00		46.00	-	47.00	54.00	29.00	Landry *et al.*, 1986
Spodoptera eridania	45.00	72.00	74.00	28.00		79.00		53.00	-	58.00	40.00	31.00	Landry *et al.*, 1986
Coleoptera													
Rhynchophorus phoenicis (Larvae)	67.33 -77.5	58.9-96.00	54.84-63.90	22.97 - 12.00	10.60	31.59-32.80	25.15-13.60	23.91-28.60	5.10	27.64-54.90	34.44	24.00	Oliveira *et al.,* 1976; Womeni *et al.*, 2012
Sciphophorus acupunctus (Larvae)	48.20	78.20	53.50	20.20	26.70	46.10	63.50	40.40	8.10	62.00	44.00	14.70	Ramos-Elorduy, 1987

Species	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Reference
Tenebrio molitor	29.00	73.00	54.00	26.00		100.00		39.00	12.00	61.00	54.00	29.00	Yi *et al.*, 2013; Hopley, 2016
Alphitobius diaperinus	43.00	66.00	61.00	26.00		120.00		39.00	12.00	58.00	54.00	34.00	Yi *et al.*, 2013
Zophobas morio	46.00	71.00	54.00	24.00		111.00		40.00	14.00	63.00	54.00	31.00	Yi *et al.*, 2013
Orthoptera													
Sphenarium purpurascens	46.00	64.00	52.00	8.00	-	36.00	32.00	49.00	10.00	54.00	42.00	21.00	Ramos-Elorduy, 1987
Acheta domesticus	36.00	66.00	53.00	25.00		92.00		35.00	9.00	55.00	65.00	21.00	Yi *et al.*, 2013
Hymenoptera													
Atta Mexicana	53.00	80.00	49.00	19.00	15.00	41.00	47.00	43.00	6.00	64.00	47.00	25.00	Ramos-Elorduy, 1987
Liometopum apiculatarum	49.00	76.00	58.00	18.00	14.00	39.00	68.00	42.00	8.00	60.00	50.00	29.00	Ramos-Elorduy, 1987
Isoptera													
Macrotermes bellicosus	51.10	78.30	54.20	7.50	18.70	43.80	30.20	27.50	14.30	73.30	69.40	51.40	Ukhun & Osasona, 1985
Macrotermes subhyalinus	37.10	79.70	35.40	12.90	9.00	43.10	36.80	41.90	7.70	51.40	-	-	Oliveira *et al.,* 1976
Hemiptera													
Corixidae	50.00	80.00	35.00	15.00	-	34.00	111.00	40.00	11.00	60.00	77.00	33.00	Ramos-Elorduy, 1987
Encosternum delegorguei	23.60	29.80	24.10	11.40	-	23.00	-	23.30	4.50	37.50	-	-	Teffo *et al.*, 2007
Blattodea													
Blaptica dubia	31.00	56.00	43.00	23.00		93.00		32.00	8.00	52.00	46.00	23.00	Yi *et al.*, 2013

*Non-essential amino acids that rely on an essential amino acid for production.

Ile-Isoleucine, Leu-Leucine, Lys-Lysine, Met-Methionine, Cys-Cysteine, Phe-Phenylalanine, Tyr-Tyrosine, Trp-Tryptophan, Val-Valine, Arg-Arginine, His-Histidine

Table 2.3 Fatty acid composition (%) of various edible insect species

Species	C14:0	C16:0	C18:0	Other SFA	Total SFA	C16:1 (n-7)	C18:1 (n-9)	Other MUFA	Total MUFA	C18:2 (n-6)	C18:3 (n-3)	Other PUFA	Total PUFA	Reference
Ledpidoptera														
Nudaurelia oyemensis	0.20	21.80	23.10	0.20	45.30	0.60	5.60	-	6.20	5.70	35.60	2.10	43.40	Bukkens, 1997
Imbrasia truncata	0.20	24.60	21.70	trace	46.50	0.20	7.40	-	7.60	7.60	36.80	-	44.40	Bukkens, 1997
Imbrasia epimethea	0.60	23.20	22.10	0.20	46.10	0.60	8.40	-	9.00	7.00	35.10	0.40	42.50	Bukkens, 1997
Imbrasia ertli	1.00	22.00	0.40	39.50	-	22.00	2.00	0.80	-	20.00	11.00	0.20	-	Oliveira *et al.,* 1976
Utsa terpsichore	2.30	27.40	0.10	37.20	-	27.40	1.70	0.20	-	27.40	2.80	0.10	-	Oliveira *et al.,* 1976
Orthoptera														
Chondacris rosea	1.09	17.24	12.42	4.36	35.25	0.78	21.12	0.30	23.14	16.46	0.62	-	41.61	Chakravorty *et al*., 2014
Brachytrupes orientalis	2.55	50.52	32.06	0.64	85.57	1.70	9.77	0.00	11.47	2.34	0.00	-	2.98	Chakravorty *et al*., 2014
Isoptera														
Macrotermes nigeriensis	0.62	31.39	7.14	-	39.35	0.62	52.45	-	53.07	7.57	-	-	7.57	Igwe *et al.*, 2012
Coleoptera														

Species	C14:0	C16:0	C18:0	Other SFA	Total SFA	C16:1 (n-7)	C18:1 (n-9)	Other MUFA	Total MUFA	C18:2 (n-6)	C18:3 (n-3)	Other PUFA	Total PUFA	Reference
Oryctes owariencis	2.50	0.20	0.23	-	3.05	-	5.24	-	43.63	45.46	4.19	-	50.86	Womeni *et al*., 2009
Homorocorphus nitidulus	0.59	0.00	0.00	-	0.59	-	6.89	-	34.75	45.63	16.19	-	62.39	Womeni *et al*., 2009

Table 2.4 Mineral composition of edible insect species (mg.100^{-1} g dry matter)

Species	Na	K	Ca	P	Fe	Mg	Zn	Cu	Mn	Reference
Ledpidoptera										
Imbrasia belina	1024.00	1032.00-1436.00	174.00-203.00	543.00-565.00	31.00-63.00	151.00-160.00	12.00-14.00	0.91-3.00	3.95	Bukkens & Paoletti, 2005
Galleria mellonella	-	-	60.00	1200	7.72	90.00	7.87	0.17	0.32-5.00	Barker *et al*., 1998; Payne *et al*., 2015
Chilecomadia moorei (larvae)	49.75	650.75	31.41	565.33	3.52	69.84	8.79	0.74	0.17	Finke, 2013
Gynanisa maia	2674.00	1467.00	112.00	563.00	9.00	167.00	23.00	3.00	1.00	Payne *et al*., 2015
Cirina forda	44.40-45.26	47.60-64.02	12.90-33.16	215.54	1.30-5.34	43.80-62.31	3.81-24.20	-	1.14	Omotoso, 2006; Osasona & Olaofe, 2010
Orthoptera										
Chondacris rosea	21.35	1130.00	340.00	-	7.81	120.00	10.83	3.62	2.76	Chakravorty *et al*., 2014

Species	Na	K	Ca	P	Fe	Mg	Zn	Cu	Mn	Reference
Brachytrupes orientalis	112.03	412.28	76.28	-	18.66	87.21	8.50	1.54	4.99	Chakravorty *et al.*, 2014
Acheta domesticus	-	-	210.00	780.00	11.20	80.00	18.6	0.85	2.96	Barker *et al.*, 1998
Coleoptera										
Tenebrio molitor	110.00	860.00	40.00-120.00	790.00-1420.00	3.99	280.00-320.00	10.10-13.10	0.21-1.70	1.75	Barker *et al.*, 1998; Hopley, 2016
Zophobas morio	100.00	760.00	60.00	490.00	5.28	110.00	6.35	0.16	1.31	Hopley, 2016
Blattodea										
Blaptica dubia	390.00	840.00	580.00	760.00	6.11	130.00	23.92	0.51	2.07	Hopley, 2016
Blaptica dubia (nymphs)	240.77	724.93	124.59	569.58	4.78	80.91	10.58	2.56	0.84	Finke, 2013
Blatta lateralis	320.00	990.00	230.00	680.00	5.57	110.00	10.73	0.62	1.45	Hopley, 2016
Naupheta cinerea	410.00	1005.00	340.00	760.00	6.85	130.00	25.16	0.59	1.33	Hopley, 2016
Gromphadorhina portentosa	410.00	1130.00	290.00	770.00	6.55	130.00	24.55	0.74	1.40	Hopley, 2016
Periplaneta americana	250.00	830.00	290.00	600.00	5.74	90.00	8.79	0.22	1.44	Hopley, 2016
Oxyhaloa deusta	300.00	960.00	250.00	680.00	5.94	130.00	30.70	0.65	1.51	Hopley, 2016

Species	Na	K	Ca	P	Fe	Mg	Zn	Cu	Mn	Reference
Diptera										
Musca domestica	824.37	127.00	41.00	240.00	27.52	115.00	32.54	1.82	34.85	Pretorius, 2011
Hermetia illucens	228.61	1167.53	2407.22	917.5	17.01	448.45	14.43	0.10	15.92	Finke, 2013
Drosophila melanogaster	-	-	140.00	1100.00	45.40	130.00	14.60	0.86	1.69	Barker *et al*., 1998
Hemiptera										
Encosternum delegorguei	2967.00	440.00	138.00	372.00	26.00	101.00	22.00	5.00	1.00	Payne *et al*., 2015
Isoptera										
Macrotermes spp.	2086.00	827.00	136.00	481.00	19.00	81.00	15.00	5.00	714.00	Payne *et al*., 2015
Macrotermes nigeriensis	112.00	336.00	0.10	1.49	0.96	6.96	0.09	0.07	0.08	Igwe *et al*., 2012

Na-Sodium, K-Potassium, Ca-Calcium, P-Phosphorus, Fe-Iron, Mg-Magnesium, Zn-Zinc, Cu-Copper, Mn-Manganese

The INN (1989) released a paper comparing the iron and calcium contents of caterpillars and beef, showing that the caterpillars contained a higher amount of both iron and calcium than beef. Various investigations have confirmed the high iron and zinc content of insect species (DeFoliart, 1992a; Barker *et al.,* 1998; Bukkens & Paoletti, 2005; Banjo *et al.,* 2006b; Pretorius, 2011; Chakravorty *et al.,* 2014; Hopley, 2016). All round, insects are nutritionally rich, with high protein and fat contents, minerals such as iron and zinc, and an amino acid profile which is superior to that of grains. These qualities are ideal for alleviating malnutrition in many African communities, and therefore more effort needs to be put into creating a comprehensive database of the nutritional aspects of edible insect species (Bukkens, 1997).

2.7 Food safety concerns of consuming insects

There are many food safety related issues that need to be addressed when considering insects as a commercial food source. There are only select insects that are edible, as many are unpalatable, poisonous or cause allergic reactions (Phillips & Burkholder, 1995; Yen, 2010; Rumpold & Schlüter, 2013a). The African silkworm pupae (*Anaphe venata)*, for example, causes thiamine deficiencies due to the presence of the enzyme thiaminase, resulting in seasonal ataxic syndromes (neurological dysfunctions) which has been an annual occurrence in Nigeria for the last 40 years (Nishimune *et al.,* 2000).

Insects are taxonomically less related to humans than animals, thereby reducing the risk of transmitting zoonotic infections (van Huis *et al.,* 2013). Furthermore, insect pathogens are taxonomically different to vertebrate pathogens, and are regarded as harmless to humans. Such is the case with the insect pathogen *Bacillus thuringiensis,* which has a different, non-overlapping life cycle compared to the human pathogen *Bacillus anthracis* (Jensen *et al.,* 1977). Currently there are no recorded cases of diseases being transmitted to humans from the consumption of insects, but with extensive farming and commercial consumption, more research will need to be done (van Huis *et al.,* 2013).

An in depth study in Nigeria by Banjo *et al.* (2006a) analysed the surface and the gut of fresh samples of the commonly eaten rhinoceros beetle (*Oryctes monoceros),* for the presence and identification of micro-organisms. Microbiological and biochemical tests revealed the growth of the food borne pathogens *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and non-pathogenic *Bacillus* species (Banjo *et al.,* 2006b). Fried beetles were also tested for microbial growth over a period of 9 days in both refrigerated and room temperature environments. The results showed a quick increase in the enumeration of bacteria when stored at room temperature, and a more gradual increase in bacterial numbers when stored in refrigerated environments (Banjo *et al.,* 2006b). A similar study by Klunder *et al.* (2012) compared the enumeration of *Enterobacteriaceae* after

three heat treatments on three different edible insects. Results showed unsafe levels of *Enterobacteriaceae* in uncooked insects, which is expected due to the soil environment in which they live (Klunder *et al.,* 2012). Boiling insects for 5 min was sufficient to eradicate *Enterobacteriaceae*, whereas roasting was not successful in eradicating all the *Enterobacteriaceae*. Blanching the insects prior to roasting proved to have favourable results, eradicating the *Enterobacteriaceae* completely. In addition to eradication of the *Enterobacteriaceae*, blanching the insects also prevented discolouration of the insects due to enzyme inactivation (Klunder *et al.,* 2012). Shelf-life tests on crickets compared the three heat treatments in various storage conditions. Results were consistent with that of Banjo *et al.* (2006a); where high microbial counts were found in the uncooked crickets, with a rapid increase in the microbial load over time, regardless of being stored under refrigerated temperatures, resulting in both visual and sensory degradation (Klunder *et al.,* 2012). Boiled crickets stored under refrigerated temperatures maintained a low count of micro-organisms for up to 2 weeks, whereas the boiled crickets stored at room temperature spoiled quickly. Dried insects, however, when stored under the same conditions, gave favourable results in both refrigerated and non-refrigerated conditions, indicating that removal of moisture is the best way to preserve insects for storage at ambient temperatures and for an extended shelf-life (Klunder *et al.,* 2012). Currently there are no regulations on the microbiological safety of edible insects, however, the Netherlands Food and Consumer Product Safety Authority have released a preliminary report with the micro-organisms which they believe to be relevant to insects and the limits of each micro-organism regarded as safe to be consumed (NVWA, 2014). The micro-organisms relevant to insects were based largely on the EU safety regulations for meat and seafood products (EU, 2005).

As is expected with any food source containing protein, there have been reports of immunoglobulin mediated allergic reactions occurring with the consumption of insects (Phillips & Burkholder, 1995; Barletta & Pini, 2003; Srinroch *et al.,* 2015). The majority of non-injected allergic reactions associated with insects are experienced by the personnel in the insect breeding facilities who are in physical contact with the insects and inhale microscopic particles of the insects on a daily basis (Phillips & Burkholder, 1995; Barletta & Pini, 2003). There have been many reports of entomologists and workers in insect breeding facilities developing allergic reactions after long term exposure to working with the insects (Barletta & Pini, 2003). Carmine dye, a red colourant derived from female cochineal insects, has been reported to cause severe allergic reactions in patients (Chung *et al.,* 2001). In China there have been two reported cases of allergic reactions from the consumption of silkworm pupae, one causing itchy sensations in the mouth and the other resulting in an anaphylactic shock (Ji *et al.,* 2008). Similarly there has been one report of a patient in Botswana being hospitalised due to an

allergic reaction when consuming the Mopane caterpillar (Okezie *et al.,* 2010). The allergic reactions induced by consuming insects are comparable to that of other arthropod species, such as crustacean allergies. The symptoms include skin rashes, congestion, and bronchial asthma. In the extreme cases, long term exposure can result in anaphylactic shock (Phillips & Burkholder, 1995; Barletta & Pini, 2003). Controlled skin sensitivity tests conducted by Bernton and Brown (1967) utilised protein extract from seven different insect species to indicate the frequency of allergic reactions. The results showed that 30 % of previously allergic patients, and 25 % of non-allergic patients showed sensitivity to at least one insect extract (Bernton & Brown, 1967; Phillips & Burkholder, 1995). The patients in this study were not accustomed to consuming insects, therefore their sensitivity was as a result of previous exposure to insect allergens either via inhalation, direct contact or accidental consumption (Bernton & Brown, 1967; Phillips & Burkholder, 1995). Heating the protein extracts at 100 °C for 1 h did not alleviate the allergic reactions and the proteins still showed positive reactions in the skin sensitivity tests (Bernton & Brown, 1967; Phillips & Burkholder, 1995).

Reese *et al.* (1999) demonstrated the cross-reactivity between allergies caused by tryptomyosin in mites and the allergies caused by tryptomyosin in shrimp. The results indicated that an individual with mite allergies could develop a sensitivity to shrimp and *vice versa* (Reese *et al.,* 1999). An in depth investigation by Srinroch *et al.* (2015) also found cross-reactivity between prawns (*Macrobrachium* spp.) and insects which was caused by the allergenic arginine kinase protein found in many varieties of prawn species and also in the field cricket (*Gryllus bimaculatus*). Previous investigations have also identified arginine kinase as an allergen in silkworm larvae (*Bombyx mori)* (Liu *et al., 2011*), the German cockroach (*Blattella germanica)* (Chuang *et al.,* 2010) and the Indian meal moth (*Plodia interpuntella*) (Binder *et al.,* 2001) Tryptomyosin and arginine kinase are both reported allergens for Arthropoda in the Allergen Nomenclature database (http://www.allergen.org/index.php) and have been found in both crustacean species and insect species, indicating that patients who are allergic to prawns or shrimp have the risk of being allergic to insect species (Srinroch *et al.,* 2015). Additionally, a novel allergen found in the field cricket, called Hexamerin1B precursor, is structurally similar to hemocyanin, an allergen commonly found in prawn species. This could also potentially result in cross-reactivity between these prawn species and the field cricket (Srinroch *et al.,* 2015).

There are concerns regarding the safety of consuming insects and these concerns need to be addressed in order to introduce insects into commercial markets. The perceived safety of consuming insects is an important factor in gaining consumer acceptance, therefore it is imperative to further investigate and understand the safety of consuming insects before advocating its consumption (Rumpold & Schlüter, 2013a; van Huis *et al.,* 2013). It has been

suggested that in order to commercialise the consumption of insects, the best approach would be to utilise commonly known edible insect species, which have been reared on pollutant-free feed in controlled farming environments as this would reduce the risks associated with eating insects (Rumpold & Schlüter, 2013b).

2.8 Functional properties

In order to incorporate insects into various food applications, it is vital to investigate and understand the functional properties of insect proteins (Rumpold & Schlüter, 2013b). The term 'functionality' has not been extensively defined, and this has led to confusion and various interpretations of the term (Hall, 1996). For clarification, in this discussion, the term 'functionality' refers to 'any property of food or food ingredients, except it's nutritional ones that influences its utilisation' such as viscosity, water and lipid absorption capacity, solubility, emulsification, gelling and foaming capacity (Hall, 1996). Typically, the functional properties of proteins contribute to creating and stabilising the characteristic structure of food products, for example, the characteristic foaming of egg whites, the curding of casein proteins in cheese and the emulsifying properties of meat proteins in sausages (Damodaran, 1994; Foegeding & Davis, 2011). Despite the hype surrounding the protein content of insects, little is known about the functional properties of these insect proteins. Yi *et al.* (2013) gave some insight into the functional properties of the protein fractions of five edible insect species, namely the gelling and foaming capabilities. Using the five species in their study as a reference point, it gives an indication that insect protein does form a foam, however, it was a weak foam, that was not particularly stable (Yi *et al.,* 2013). In many African countries insects are dried and ground into flour (Osasona & Olaofe, 2010; Womeni *et al.,* 2012; Assielou *et al.,* 2015) and investigations into the foaming capabilities of insect flour found *O. owariensis* larvae flour to have poor foaming capacities (Assielou *et al.,* 2015) and *C. forda* flour to have no foaming capacity at all (Osasona & Olaofe, 2010). In terms of gelation, insect protein fractions have shown strong gelling capabilities at a 15- 30 % $w.v^{-1}$ concentration, with the house cricket protein creating a particularly strong gel (Yi *et al.,* 2013). Similarly, *O. owariensis* larvae flour and *C. forda* flour were found to have good gelling properties (Omotoso, 2006; Osasona & Olaofe, 2010). Insect flour typically has high water and lipid absorption capacities (Omotoso, 2006; Osasona & Olaofe, 2010; Womeni *et al.,* 2012; Assielou *et al.,* 2015), as well as, good emulsifying activities (Assielou *et al.,* 2015; Omotoso, 2006; Osasona & Olaofe, 2010), suggesting that they would be ideal as texturing ingredients and flavour retainers. These results suggest that insect proteins do have the potential to become a functional ingredient, however, more research needs to be done on other functional properties and on different insect species, in various processing conditions, in order to develop processing techniques specific to insects/insect species.

2.9 Western ideas and attitudes

The traditional practice of entomophagy has decreased over the years due to the globalisation of Western ideas, religions and lifestyles (DeFoliart, 1999; Premalatha *et al.,* 2011; Meyer-Rochow & Chakravorty, 2013). In Malawi, for example, Christianity is followed due to the travelling missionaries, and the people of Malawi no longer eat insects, as it has been condemned by the local church as a heathen custom (Silow, 1983). The Western influence and their feelings of disgust towards consuming insects remains the largest barrier preventing the acceptance and commercial consumption of edible insects (Verkerk *et al.,* 2007; Hartmann *et al.,* 2015). De Foliart (1999) was quoted saying "Westerners should become aware of the fact that their bias against insects as food has an adverse impact, resulting in a gradual reduction in the use of insects without replacement of lost nutrition and other benefits." In cultures where insects are traditionally eaten, people are more likely to adopt the practice of entomophagy, even if they themselves are not regular consumers of insects. This is because they have been exposed to insects as a food source, and the idea of consuming insects is set in their cultural consciousness (Hartmann *et al.,* 2015; Tan *et al.,* 2015). In Western culture, however, insects have not been a part of the cultural diet, resulting in more of an aversion towards incorporating them into their diet (Hartmann *et al.,* 2015; Tan *et al.,* 2015). Food neophobia is a term used to describe someone's reluctance to try or consume foreign or novel foods (Al-Shawaf *et al.,* 2015; Verbeke, 2015), and plays a significant role in their willingness to accept insects as a food source (Verbeke, 2015).

The concept of entomophagy is gaining momentum, and the Western culture is beginning to slowly accept insects as a food source. Recent investigations have explored the reasons and psychology behind the Western consumers' reluctance to eat insects, with the main focus being on ways to try and change this mind-set. During multiple focus group interviews conducted in both the Netherlands and Thailand, the effect of cultural exposure and individual exposure to entomophagy was explored and it was established that cultural and individual experience had a significant effect on their acceptance of insects as a food source (Tan *et al.,* 2015). This was observed when Thai participants were repulsed by the mealworms, as they were unfamiliar with eating them and associated them with decaying matter, whereas the Dutch participants preferred products with mealworms, as they were more familiar with them (Tan *et al.,* 2015). Cultural exposure also had a significant impact on the reasoning for participants consuming insects. The Thai participants, having been exposed to consuming insects in their culture, embraced the idea because of its familiarity and taste, whereas the Dutch participants, having no cultural background in entomophagy, considered eating insects as it has been promoted as a sustainable protein source and because of its novelty (Tan *et al.,* 2015). Individual experiences also had a significant effect on their

willingness to try an insect product. Thai participants who consumed insects regularly were more willing to consume both the insects themselves, and the insect products available in the study, however, the Thai participants who disliked eating insects due to previous bad experiences with insect consumption were more hesitant to trying them (Tan *et al.,* 2015). This was also observed in the Dutch participants, most of whom were intrigued and tasted some of the insect products, however, they were averse to consuming insects when they were easily visible. Having grown up in a society where the consumption of insects is not common place, Dutch participants were not accustomed to seeing whole insects in meals, and therefore preferred the meals or products where they were disguised or blended within the product (Tan *et al.,* 2015). Three other studies had similar findings and it was established that products with visible insects were met with more disgust and apprehension by Western consumers, than those where insects are processed and disguised in the product (Schösler *et al.,* 2012; Hartmann *et al.,* 2015; Gmuer *et al.,* 2016). Aside from the lack of cultural and individual experience of eating insects in Western societies, Western consumers are not accustomed to consuming animals when seeing their whole form, as it aids as a reminder that the animal was once alive and triggers a negative response towards consuming the meat (Rozin & Fallon, 1987). This same psychology also explains why Western consumers would prefer consuming insects that are disguised in a product as oppose to present in a meal in their whole form (Hartmann *et al.,* 2015). It was also found that Western consumers were more likely to consume insects in meals or products that they were more familiar with, as opposed to new or unknown products containing insects (Tan *et al.,* 2015). Interestingly, the same applied to Thai consumers, who preferred eating insects in their whole form as they were accustomed to, as opposed to consuming insects disguised in processed products (Tan *et al.,* 2015). These studies suggested that insects should be incorporated into familiar food products in order to introduce insects as a food source into Western societies (Schösler *et al.,* 2012; Hartmann *et al.,* 2015; Tan *et al.,* 2015). A good example of this is insect burger patties, which are currently being sold in niche markets in Europe. Blind tastings revealed that consumers thought the insect patties compared well to commercial vegetarian patties, however, they were rated poorly in terms of texture and juiciness when compared to a commercial meat patty (Schouteten *et al.,* 2016).

At this point in our history, consuming insects may be a controversial topic in the Western culture, however, cultural taste and preferences can be changed. A good example of this is the worldwide prevalence of sushi (raw fish) which was previously not considered palatable by the Western world and suddenly became one the trendiest food items on the menu (Johnson, 2010). Similarly, lobster, a family member of insects, was once regarded as unpalatable and only fed to prisoners at times of food shortages, and it is now also considered

a delicacy in Western culture (Townsend, 2012). With such examples, it begs the question as to why insects have not been fully accepted by the Western culture despite the efforts being made to promote entomophagy. Shelomi (2015) discussed the latter with reference to relative advantage, compatibility, trialability, complexity and observability. Currently the focus is on the novelty of insects, however, it is suggested that the marketing strategy should be shifted from a novelty food to one where the concept is portrayed as a normal and an everyday food (Shelomi, 2015). Along with this, the focus should be on creating and maintaining a constant, inexpensive supply of insects and insect products, and making obtaining them readily available for consumers to try at will (Shelomi, 2015). Globally there needs to be a strong drive to reduce their negative stigma in the Western culture in order for research to be conducted and systems also need to be implemented to mass-rear insects for commercial production (DeFoliart, 1999). Effort must be made to try and change these preconceived ideas consumers have towards the sensory properties of insects, by taste exposure (Tan, 2016). To encourage regular consumption of insects, it is important that they are incorporated into appropriate food products that would still be appealing to consume once the novelty has worn off (Tan, 2016). Consumers are prone to liking insect products more once informed about their good nutritional profile and sustainability (Schouteten *et al.,* 2016; Verneau *et al.,* 2016), however, ultimately it is more important for the insect to be considered appropriate by the consumer (Tan *et al.,* 2016), and for the sensory properties of the products be desirable (Schouteten *et al.,* 2016; Tan *et al.,* 2016) as this will increase the chances of consumers repeatedly buying the product. Ramos Elorduy (1990) holds researchers responsible by saying “Insects have long been a significant dietary factor in the poorer regions of the world, and it is high time that scientists recognise this fact and begin to build on it, rather than discouraging or ignoring the practise”. The responsibility falls upon the shoulders of the food industry to promote the consumption of insects by creating tasty products and dishes targeting a more mainstream market (van Huis *et al.,* 2013). Having insects that are readily available for consumers will create the demand that scientists are waiting for (Shelomi, 2015).

2.10 Concluding remarks

The use of insects as a food source has the potential to provide a sustainable, nutritionally rich protein alternative. Currently insect consumption is a novelty, with very few commercial products to choose from. Therefore, the first step is to optimise the farming of insects and to create a cheap, continuous supply of insects that will make the development and consumption of insects possible. To create a global culture that accepts and enjoys entomophagy, there needs to be a strong drive to educate consumers, not only on the nutritional and sustainable benefits of consuming insects, but on the desirable sensory attributes it can impart to food products. The functional properties of various insect species needs to be understood in order

to optimise processing parameters to ensure products of good quality and sensory properties can be produced. The food safety of consuming insects and insect products needs to be fully investigated before fully advocating its consumption, which also ensures that hygienic and safe processing procedures are developed. It is the hope that increasing the acceptance of entomophagy in the Western culture will result in an increase in entomophagy in cultures that are less affluent (Shelomi, 2015).

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Chapter 3

Determining the proximate, amino acid and fatty acid composition of blanched and unblanched *Tenebrio molitor*, *Blatta lateralis*, *Blaptica dubia*, *Hermetia illucens* and *Naupheta cinerea*, as well as, the microbial load of *T. molitor* and *H. illucens* larvae.

3.1 Abstract

The proximate composition and chemical composition of five blanched and unblanched edible insect species, namely *Tenebrio molitor* (mealworm) larvae, *Blatta lateralis* (Turkistan roach), *Blaptica dubia* (orange spot roach), *Hermetia illucens* (black soldier fly) larvae and *Naupheta cinerea* (lobster cockroach) were determined, as well as, the microbial load of *T. molitor* and *H. illucens* larvae. *Tenebrio molitor* had the highest fat content (35.7 g.100g^{-1}), whilst *B. lateralis* had the highest protein content (101.5 g.100g^{-1}). The insects all had similar energy values averaging 24.12 MJ.kg^{-1}. The crude fibre ranged from 8.7 g.100g^{-1} in *T. molitor* to 19.1 g.100g^{-1} in *H. illucens*. All of the insects were good sources of minerals, specifically iron and zinc, which were higher than conventional sources. The amino acid profile of each insect species compared favourably to the daily requirement for an average adult, with the exception of methionine, which was the limiting amino acid in all of the insects tested. The fatty acid content differed slightly between insect species, however, oleic acid was the most prominent fatty acid in all of the insects tested, with values ranging from 11.6 % in blanched *H. illucens* to 46.2 % in *B. dubia*. Linoleic acid was the most prominent polyunsaturated fatty acid with contents ranging from 3.3 % in the blanched *H. illucens* to 13.9 % in the blanched *B. lateralis*. Overall, these insects were considered good sources of protein, fat, energy, minerals, amino acids and fatty acids. With regard to microbial safety, both *T. molitor* and *H. illucens* contained high Total Viable Count (TVC) and *Enterobacteriaceae,* however, blanching the *T. molitor* and *H. illucens* reduced the levels to less than10 cfu.g^{-1}. The aerobic endospore count was low on both *T. molitor* and *H. illucens,* and Salmonella was not found on either insect species. There was some growth of *Listeria* species (<5 cfu.g^{-1}) on unblanched samples, which could be a potential problem. Overall, blanching is suggested prior to eating, as it significantly reduced all microbial levels to safe levels for human consumption.

Keywords: Edible insects, alternative food source, nutritional value, food safety.

3.2 Introduction

The practise of consuming insects, known as entomophagy, forms an important part of over 2 billion people's diet worldwide. A reported 2037 insect species are eaten globally (Jongema, 2015), with a large portion of those people situated in various African countries. In Africa, a variety of insects are considered delicacies and fetch higher prices in the markets than conventional meat (Parent & Thoen, 1977; Kozanayi & Frost, 2002; Stack *et al.,* 2003). Mopane caterpillars (*Imbresia belina*), endemic to Southern Africa, are highly sought after throughout the African continent and are a good source of protein and energy, with protein contents of 48 - 56 % and energy contents of 14 -18 $MJ.kg^{-1}$ (Bukkens, 1997; Ghaly, 2009).

Insects as a food source has become appealing due to their high reproductive rate and feed conversion efficiency, their good nutritional profile, and their potential to be a sustainable food source by recycling waste matter and using very little space and water (Aarnink *et al.,* 1995; Oonincx *et al.,* 2010). The majority of the information published concerning edible insects revolves around their desirable nutritional composition. Edible insect species have shown favourable results with regard to their protein, fat, amino acid, fatty acid and mineral content. The protein content of many insect species are comparable to beef and pork, and their zinc and iron contents are superior to conventional animal and plant sources (Bukkens & Paoletti, 2005; Chakravorty *et al.,* 2014). The amino acid profile of most insect species are desirable and can complement the high cereal grain, low lysine diet in most developing countries. It has been suggested that consuming insects can reduce the incidences of malnutrition in impoverished areas, and contribute to food security in many developing countries (Bukkens, 1997; Moreki *et al.,* 2012). Factors such as species, diet, environment and life stage have been found to affect the nutritional profile of insects (Omotoso & Adedire, 2007; Ademolu *et al.,* 2010; Xiaoming *et al.,* 2010; Oonincx, 2015).

Despite the age old tradition of consuming insects, Western culture still frowns upon the consumption of insects. However, the many benefits of consuming insects have begun to slowly overshadow feelings of disgust, creating an increasing interest in utilising insects as a food source for humans (Rumpold & Schlüter, 2013; van Huis *et al.,* 2013). With the recent increase in interest, it is vital to obtain information on the nutritional profile of various edible insect species, as well as, the safety of consuming them. The perceived safety of consuming insects is an important factor in gaining consumer acceptance, therefore it is imperative to investigate and understand the safety of consuming insects before advocating its consumption (van Huis *et al.,* 2013). High microbial loads have been found on various insect species, and it is suggested that blanching be employed as a pre-treatment to ensure microbial safety (Banjo *et al.,* 2006a; Klunder *et al.,* 2012). It was therefore the objective of this study to determine the proximate and chemical composition of five blanched and unblanched, edible

insect species. According to the European Union, both *T. molitor* and *H. illucens* are considered to have the biggest potential to be used in food and feed (EFSA Scientific Committee, 2015), therefore the microbial safety of unblanched, purged and blanched *T. molitor* and *H. illucens* were determined with the aim being to advise on any potential health implications of consuming *T. molitor* and *H. illucens*.

3.3 Materials and Methods

3.3.1 Sample preparation

In total 50 g of each of the five insect species (Fig. 3.1), namely *Tenebrio molitor* (mealworm) larvae, *Blatta lateralis* (Turkistan roach), *Blaptica dubia* (orange spot roach), *Hermetia illucens* (black soldier fly) larvae and *Naupheta cinerea* (lobster cockroach), were randomly sampled in triplicate from the insect farms at the Animal Sciences Department at Stellenbosch University. The insects were fasted for 24 h to clear their gastrointestinal tract, and then killed by freezing them at -20 °C for 24 h. Upon thawing, half of the insects were blanched at ± 98 °C for 2 min and the other half remained unblanched. The insects were dried and prepared for analysis as described by Hopley (2016).

Figure 3.1 From top left to right - *Tenebrio molitor* (www.wildmanfoods.org), *Blatta lateralis* (www.mantidkingdom.com), *Blaptica dubia* (www.DubiaRoaches.com), *Hermetia illucens* (http://www.agriprotein.com) and *Naupheta cinerea* (www.exotic-pets.co.uk).

3.3.2 Proximate analysis

3.3.2.1. Moisture content

Moisture content was determined according to Official AOAC Method 934.01 (AOAC, 2002). Each sample (2.5 g) was placed in a pre-dried crucible and dried at 100 °C for 24 h. Once cooled in a desiccator, the crucibles containing the dried sample were weighed, and the moisture content was calculated using equation 3.1.

% Moisture = $\frac{(A+B)-C}{B}\ x\ 100$ **(Equation 3.1)**

Where:

A= Weight of dried crucible; B=Weight of wet sample; C=Weight of crucible containing dried sample

3.3.2.2. Ash

The ash content was determined according to Official AOAC Method 942.05 (AOAC, 2002). The crucibles containing the samples were placed in a furnace at 500 °C for 6 h. Once removed and cooled in a desiccator, the crucibles containing the sample were weighed, and the ash content was calculated using equation 3.2.

% Ash= $\frac{D-A}{E}\ x\ 100$ **(Equation 3.2)**

Where:

A= Weight of dried crucible; D=Weight of crucible and ash; E=Sample mass

3.3.2.3. Crude fat

The crude fat was determined on the dried samples (2 g) using two methods, as there are no standard methods for testing insects.

Acid hydrolysis (AOAC Method 954.02): 2 ml of ethanol and 10 ml HCl was added to the tube with the sample and boiled for 30 min in a water bath. Once removed and cooled, they were poured into a separating funnel and the tube was rinsed with 10 ml ethanol. Thereafter, 25 ml of diethyl ether was added and shaken for 1 min, and then 25 ml of petroleum ether was added and shaken for 1 min. The upper portion of the liquid in the separating funnel was poured into a fat cup. This process was repeated twice more. The fat cups were placed in a 100 °C oven for 2 h, cooled and then weighed. The fat content was calculated according to equation 3.3 (AOAC, 2002).

Soxhlet extraction (AOAC Method 920.39): samples were weighed into dry thimbles, covered with cotton wool and placed in the Tecator Soxtec System HT 1043 Extraction Unit

(Velp Scientifica; Trident Instrumentation, Tyger Falls Boulevard, Cape Town, South Africa). Thereafter, 50 ml of diethyl ether was added to the pre-weighed, dried aluminium fat cups and placed in the extraction unit. The thimbles were dropped into the diethyl ether for 15 min to boil, and then lifted for 30 min. Thereafter the taps were closed for 15 min, while the boiling continued. The aluminium cups were removed and placed in the drying oven for 2 h. Once removed and cooled, the beakers were weighed and the fat content was calculated according to equation 3.3 (AOAC, 2002).

Fat % = $\frac{F-A}{E} \boldsymbol{x\ 100}$ **(Equation 3.3)**

Where:

A=Weight of dried fat cup; E=Weight of sample; F= Weight of fat cup plus fat

3.3.2.4. Crude Protein

The crude protein was determined by determining the total nitrogen content using the LECO FP528 analyser (LECO Africa, Kempton Park, South Africa) according to Official AOAC Method 992.15 (AOAC, 1992). EDTA was used to calibrate the LECO analyser. Each dried sample (0.100 g) was weighed into a tin cup and then placed in the LECO analyser. The result displayed the percentage nitrogen which was multiplied by a factor of 6.25 to obtain the percentage protein.

3.3.2.5. Crude Fibre

The crude fibre was determined using the Fibretec analyser FIWE6 (Velp Scientifica; Trident Instrumentation, Tyger Falls Boulevard Waterfront, Cape Town, South Africa) according to Official AOAC Method 962.09 (AOAC, 2002). Dried sample (1 g) was weighed into a pre-dried glass crucible and placed into the Fibretec hot extraction unit. The samples were defatted using acetone. The samples were boiled in 0.128 M sulphuric acid, followed by boiling in 0.313 M sodium hydroxide. The crucibles were then dried for 24 h at 100 °C, weighed and then ashed in the furnace for 6 h at 500 °C, and weighed once again. The crude fibre was calculated using equation 3.4.

% Crude fibre = $\frac{A-B}{E} \boldsymbol{x\ 100}$ **(Equation 3.4)**

Where:

A = Weight of residue in crucible after drying; B = Weight of residue in crucible after ashing; E=Weight of sample.

3.3.2.6. Minerals

The five insect species, as well as, the blanched samples were dried, milled, and then sent to Elsenburg (Muldersvlei Road, Elsenburg, Stellenbosch, 7607) where the mineral analyses was done. The minerals phosphor (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), borium (B) and aluminium (Al) were analysed using the combustion method 6.1.1 as described in the Handbook of feeds and plant analysis (ALASA, 1998) and measured on a iCAP 6000 series Inductive Coupled Plasma Spectrophotometer. Mineral concentrations were calculated in $mg.100g^{-1}$ using the iTEVA Analyst software.

3.3.2.7. Total Energy

The total energy was determined by measuring the amount of heat produced when the samples were completely oxidized in the C200 Bomb Calorimeter (Somerset Business Park, Somerset West, South Africa). Benzoic acid tablets were used to calibrate the bomb calorimeter. A 0.3 g - 0.5 g dried sample was made into a pill, weighed and then placed directly on top of the cotton thread inside the metal crucible. The crucible was placed inside the decomposition vessel and closed. The vessel was filled with 3 bar of oxygen and then placed into the inner vessel of the calorimeter. Two litres of tap water was poured into the tank, the weight of the sample was inserted into the calorimeter, the lid was closed and the system was fired. The result displayed was in $MJ.kg^{-1}$.

3.3.3 Fatty Acid Determination (FAME method)

Fatty acids were determined using gas chromatography (GC). The lipids were extracted by adding 0.5 g of dried sample to a 2chloroform:1methanol (Sigma Aldrich Inc., 3050 Spruce str., St. Louis, MO, 63103, USA, Cat no. H3500, 98 %) solution containing 0.01 % Butylated hydroxytoluene (BHT) (Cat no. B-1378, Sigma Aldrich) and Heptadecanoic acid (C17) as an internal standard. The samples were homogenised with the Polytron (Kinematica AG PT 2500 E, speed 7-8 x 1000 rpm) for 40 s and then transferred to an extraction funnel through a glass microfiber filter paper. The extraction tubes were rinsed with 20 ml of CM 2:1 and added to the extraction funnel, where the funnel was then dried using a vacuum. Once filtration was completed, the volumetric flask was filled up to 50 ml with Chloroform/Methanol (CM) 2:1 (Folch *et al.,* 1957). The solution (250 µl) from the volumetric flask was transferred to a Klimax tube and completely dried in a 45 °C water bath under nitrogen. Once dried 2 ml of transmethylating reagent (19:1 methanol:sulphuric acid) was added, vortexed and then sealed. The Klimax tubes were once again placed in a 70 °C water bath for 2 h, then removed and cooled. Distilled water (1 ml) and hexane (2 ml) was added, vortexed, and allowed to

settle. The top phase of liquid was transferred to a new Klimax tube and completely dried under nitrogen in a 45 °C water bath. Thereafter, 100 µl hexane was added to the dry sample, vortexed and transferred into a GC vial containing an insert. The samples were then loaded into the GC (Trace 1300 Series; Thermo Scientific; S/N 712100906; Thermo Fisher Scientific S.p.A.; Strada Rivoltana; 20090 Rodano, Milan, Italy) equipped with the GC auto-sampler (CTC Analytics, Combi PAL, Product No: G6500-CTC, Serial No: CH00127681, Waldbronn, Germany) and the GC column (TR FAME, Length: 30 m; internal diameter: 0.25 mm, Film: 0.25 um, P/N 260M142P). The specified conditions were as follows: carrier gas used was hydrogen (0.7 ml·min^{-1}), the initial temperature was 50 °C, the final temperature was 240 °C, the injector temperature was 250 °C and the detector temperature was 280 °C. The rate of temperature increase was 12 °C per min.

3.3.4 Amino Acid Determination

Amino acid hydrolysis was determined according to AOAC official method 994.12 (2003). Dried samples (0.1 g) were placed in a hydrolysis tube, and hydrolysed with 6 ml 6 N HCL and 13 % Phenol, then sealed under a vacuum, and placed in an oven at 110 °C for 24 h. Once removed and cooled after 24 h, the contents were poured into Eppendorf tubes and taken to the LSMS laboratory (Room 225, JC Smuts Building, Van Der Byl Street, Stellenbosch, 7600) for derivatisation and identification using High Performance Liquid Chromatography (Waters AccQ Tag Ultra Derivatization Kit; AccQ Tag C17 coloumn, 1.7 µm, 2.1 x 100 mm).

3.3.5 Microbiological tests

Hermetia illucens and *T. molitor* were randomly sampled from the insect farms at the Animal Sciences Department at Stellenbosch University, and tested at three different stages, namely at harvest, after being purged for 24 h, and after blanching at 98 °C for 3 min. *Hermetia illucens* and *T. molitor* were rinsed with a saline solution, and then distilled water to remove any dirt or contamination from the farming environment.

For each test 10 g of the insects sampled was added to 90 ml of autoclaved Physiological Salt Solution (PSS) in a stomacher bag, and then stomached for 2 min (Seward stomacher 400). A dilutions series (10^{-1} to 10^{-5}) of the product-PSS solution was prepared and used for each test. All agar and solutions were prepared according to manufacturer's specifications (Merck, South Africa). Total viable count (TVC) was determined by spread plate on Tryptic Soy Agar (TSA) (Merck, South Africa), and then incubated at 37 °C for 48 h (da Silva *et al.,* 2012; SANS, 2012). Aerobic endospores were tested by heating the sample to 75 °C for 20 min, and then spread plating it on TSA agar (Merck, South Africa) and incubating it at 35 °C for 48 h (Austin, 1998; da Silva *et al.,* 2012). Enumeration of *Enterobaceriaceae* was

tested by spread plate technique on Violet Red Bile Agar (VRBA) (Merck, South Africa) and incubated at 30 °C for 24 h (Merck, 2007; da Silva *et al.,* 2012).

The presence of *Listeria monocytogenes* was tested using a Fraser and half Fraser broth. Firstly, 25 g of each treatment was added to 225 ml of half strength Fraser broth and incubated at 35 °C for 24 h (SANS, 2001). Thereafter 0.1 ml of each incubated sample was transferred into 10 ml of full Fraser broth and incubated at 35 °C for 48 h (SANS, 2001). The half Fraser broth culture was then streaked onto Oxford agar plates (Merck, South Africa), and PALCAM agar plates (Merck, South Africa) and incubated micro-aerobically at 35 °C for 24 h (SANS, 2001). The same was performed with the culture from the full Fraser broth (SANS, 2001).

The presence of *Salmonella* was tested in four successive stages. Firstly 25 g of each sample was added to Buffered Peptone Water (BPW) and incubated at 35 °C for 24 h (SANS, 2003; da Silva *et al.,* 2012). Thereafter, 0.1 ml of the incubated samples were transferred to 10 ml of *Salmonella* enrichment broth (Merck, South Africa) and incubated for 42 °C for 24 h, and then for a further 24 h at 35 °C (SANS, 2003; da Silva *et al.,* 2012). The inoculated *Salmonella* enrichment broth samples were then streaked out on Xylose Lysine Deoxycholate (XLD) agar (Merck, South Africa) agar plates and incubated at 35 °C for 24 h (SANS, 2003; da Silva *et al.,* 2012).

3.4 Results and Discussion

There was no conclusive evidence to suggest that blanching the samples either destroyed or enhanced the nutritional value of the insects, however, it was evident that blanching the insects aided in colour retention. A similar effect was observed by Klunder *et al* (2012), where it was speculated that the colour retention was caused by denaturation of the enzymes. Photos were taken of the unblanched and blanched samples after milling to demonstrate the colour differences (Fig. 3.2). The time taken for insect samples to discolour was seen to be approximately 2 min.

Figure 3.2 Photos taken after milling showing the colour differences between unblanched samples (left) and blanched samples (right).

3.4.1. Proximate Analysis

The moisture content of the insects ranged from 61.83 g.100g^{-1} in the lobster cockroach to 77.48 g.100g^{-1} in the blanched *H. illucens* (Table 3.1). As expected, the blanched insects had higher moisture contents than their non-blanched counterparts, due to the absorption of water upon blanching. The ash content ranged from 1.35 g.100 g^{-1} in *N. cinerea* to 2.93 g.100g^{-1} in the blanched *H. illucens* sample.

Table 3.1 Proximate analysis of five edible insect species on a dry matter basis g.100g^{-1} (DM)

	Moisture	Crude Protein	Crude Fat (Soxhlet)	Crude Fat (Acid Hydrolysis)	Crude Fibre	Ash	Total Energy (MJ.kg^{-1})
N. cinerea	61.83 ± 0.10	77.29 ± 0.36	15.22 ± 0.02	14.94 ± 0.34	13.56 ± 0.04	1.35 ± 0.03	22.72 ± 0.54
Blanch *N. cinerea*	72.93 ± 0.68	74.38 ± 0.00	17.98 ± 0.46	14.13 ± 0.39	12.68 ± 0.33	1.78 ± 0.03	21.93 ± 0.34
B. lateralis	74.17 ± 1.71	101.46 ± 1.80	7.45 ± 0.31	15.58 ± 0.22	9.09 ± 0.21	2.03 ± 0.02	22.23 ± 0.02
Blanch *B. lateralis*	74.69 ± 0.96	99.17 ± 1.30	7.43 ± 0.04	16.65 ± 0.15	9.92 ± 0.08	1.58 ± 0.04	21.87 ± 0.02
T. molitor	58.23 ± 1.01	49.00 ± 0.10	29.21 ± 0.57	35.75 ± 0.91	8.66 ± 0.12	1.77 ± 0.05	25.26 ± 0.28
Blanch *T. molitor*	62.96 ± 0.39	48.17 ± 0.65	29.17 ± 0.83	34.033 ± 0.41	12.65 ± 0.19	1.66 ± 0.04	25.25 ± 0.35
B. dubia	66.86 ± 0.27	60.98 ± 0.24	21.99 ± 0.55	22.86 ± 0.28	11.36± 0.34	1.91 ± 0.01	23.11 ± 0.43
Blanch *B. dubia*	69.46 ± 0.68	66.46 ± 3.44	24.24 ± 0.14	22.31 ± 0.58	14.39 ± 0.29	1.48 ± 0.01	24.73 ± 0.73
H. illucens	75.8 ± 0.19	45.60 ± 0.93	18.734 ± 0.12	18.01± 0.24	19.14 ± 0.11	2.69 ± 0.04	28.07 ± 0.29
Blanch *H. illucens*	77.48 ± 0.44	45.79 ± 0.42	22.81 ± 0.88	18.75 ± 0.57	18.97 ± 0.66	2.93 ± 0.01	26.06 ± 0.51

The fat content from the Soxhlet extraction ranged from 7.43 g.100g^{-1} in blanched *B. lateralis* to 29.21 g.100g^{-1} in *T. molitor*. Acid Hydrolysis seemed to extract more fat with fat contents ranging from 14.13 g.100g^{-1} in blanched *B. lateralis* to 35.78 g.100g^{-1} in *T. molitor*. The protein content of insects is of particular interest, as insects are considered to have the potential to alleviate protein deficiencies in developing countries. The crude protein was the largest portion of the nutritional composition and on a dry matter basis the contents ranged from 45.60 g.100g^{-1} in *H. illucens* to 101.46 g.100g^{-1} in *B. lateralis.* Therefore, 100 g of dried insects can contribute to 91.2 % and 203 % of the recommended daily requirement (50 g.d^{-1}) respectively (Food and Drug Administration, 2014). The protein content of insects are generally comparable with that of beef and pork (DeFoliart, 1992; Bukkens & Paoletti, 2005; Chakravorty *et al.,* 2014), and this was also found to be true when comparing the protein contents of the insects in this study to conventional protein sources (Fig. 3.3) (USDA, 2016). The protein contents of the insects tested were superior to both soy and chicken, and only T. molitor and *H. illucens* were slightly less than beef and pork, whilst the roaches had protein contents that were comparable to both beef and pork (USDA, 2016). In this investigation, the nitrogen content was multiplied by a factor of 6.25 to calculate the protein content, however, more research is required that can accurately identify and determine the amino acids concentrations to derive a factor suitable for insects. It may be the case that different factors will be relevant to different insect species depending on their chitin content. It is suggested that a factor of 6.25 is too high and could explain the crude protein of more than 100g.100g^{-1} sample (Bukkens, 1997).

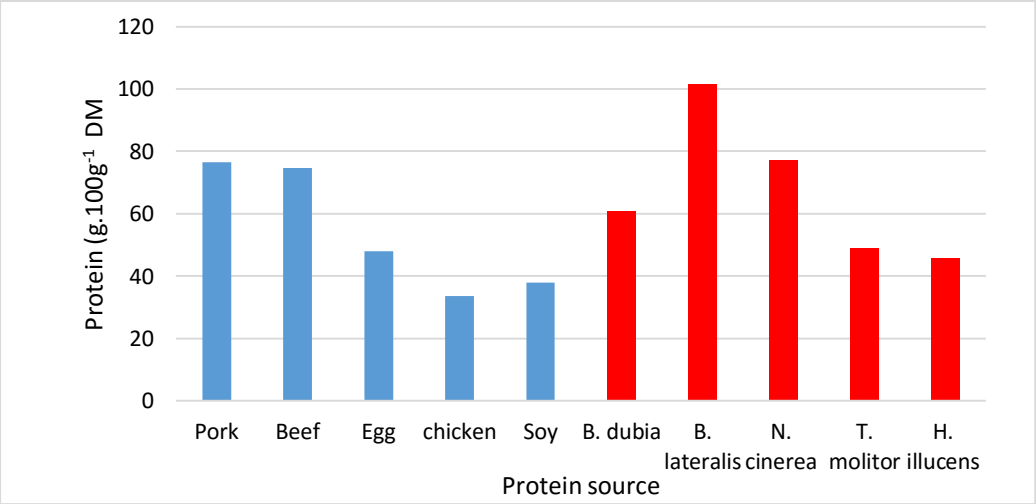


Figure 3.3 Protein content of five edible insect species ((*Tenebrio molitor*, *Blatta lateralis*, *Blaptica dubia*, *Hermetia illucens* and *Naupheta cinerea*) compared to some conventional plant and animal protein sources (adapted from USDA, 2016).

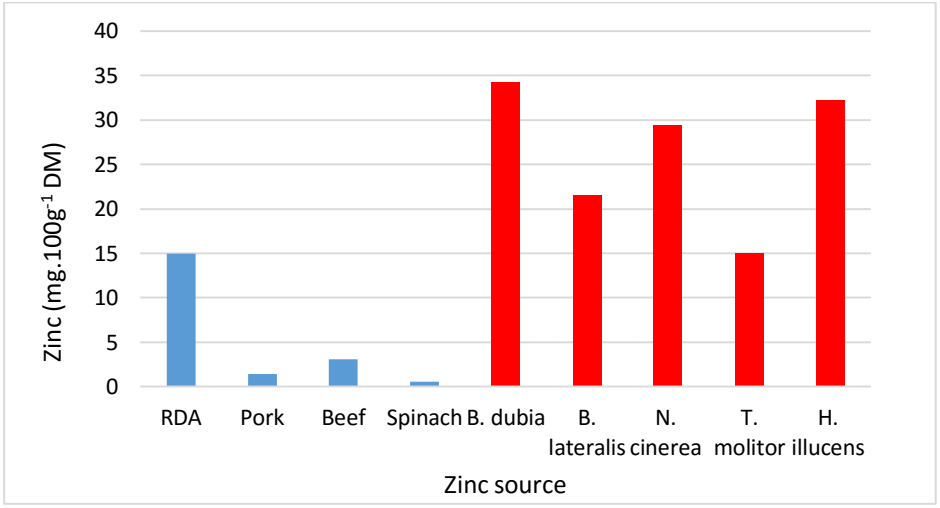
The crude fibre ranged from 8.66 g.100 g^{-1} in *T. molitor* to 19.14 g.100 g^{-1} in *H. illucens* (Table 3.1). The crude fibre content has been attributed to the chitin content of the insects. Chitin is the primary component in the exoskeleton of insects, and it is a fibre, consisting of long-chain N-acetylglucosamine groups and is a derivative of glucose (Goodman, 1989; Goosen, 1996). Insects with a hard exoskeleton are thought to have higher chitin contents and therefore a higher crude fibre content (Bukkens, 1997), however, this was disproved by Finke (2007). In this investigation, *H. illucens* had the highest crude fibre content of 19.2 g.100g^{-1} (Table 3.1), and their exoskeleton is not as hard as that of the cockroaches, which is in agreement with the findings by Finke (2007).

In many developing countries calorie deficiencies are one of the main contributors to malnutrition (DeFoliart, 1992). Insects are considered to be a good source of energy and the total energy value of the insects ranged from 21.93 MJ.kg^{-1} in blanched *B. lateralis* to 28.07 MJ.kg^{-1} in *H. illucens* (Table 3.1). The energy contents of *T. molitor*, *B. dubia* and *B. lateralis* correspond well to that reported by Hopley (2016). *H. illucens* and *B. lateralis* had higher energy values in this study when compared to results presented by Finke (2013). Finke calculated the total energy using standard calculations as opposed to instrumental analysis, which can allow for discrepancies in their results (Finke, 2013). Overall, the results of the analysis in this study corresponds well to that of previous research on *B. dubia* (Yi *et al.,* 2013) and *T. molitor* (Ghaly & Alkoaik, 2009; Yi *et al.,* 2013; Hopley, 2016). The nutritional composition of *H. illucens* was slightly inconsistent with a previous study by Finke (2013), where only the protein values were comparable. *B. lateralis* and *N. cinerea* had higher protein, fibre and ash contents and lower fat contents than previously reported (Hopley, 2016). The slight variability in the proximate analysis between studies can be attributed to variety in the diet, as well as, the age when the insects/larvae were harvested (Landry *et al.,* 1986; Xiaoming *et al.,* 2010; Ademolu *et al.,* 2010).

Minerals are an important part of the human diet, with great importance being placed on iron, zinc and calcium intake (FAO & WHO, 2005). Iron deficiency (anaemia) is one of the most common nutritional disorders across the globe, particularly in developing countries (WHO, 2001; FAO & WHO, 2005). Zinc deficiency is another common mineral deficiency which causes many deaths in developing countries each year (De Benoist *et al.,* 2007). It has been established that insects have high contents of iron, making them a desirable food source for malnourished communities (DeFoliart, 1992; Banjo *et al.,* 2006b; Bukkens & Paoletti, 2005; Chakravorty *et al.,* 2014). The insects in this study had iron contents ranging from 5.76 mg.g^{-1} in blanched *T. molitor* to 32.56 mg.g^{-1} in *H. illucens* (Table 3.2). Consuming 100 g of *H. illucens* would contribute to nearly double the amount of what the average human requires (18 mg.d^{-1}) (Food and Drug Administration, 2014). The high iron values of the *H. illucens* are

similar to the values found in the Mopane caterpillar (*Imbresia belina)*, which is a popular food source in many Southern African countries (Bukkens & Paoletti, 2005; Ghaly, 2009). Insects in general have also been documented to have high zinc contents (Barker *et al.,* 1998; Bukkens & Paoletti, 2005; Pretorius, 2011; Hopley, 2016). The zinc contents ranged from 14.93 $mg.g^{-1}$ in blanched *T. molitor* to 38.20 $mg.g^{-1}$ in blanched *B. dubia* (Table 3.2), all of which are higher than the recommended 15 mg daily intake for the average adult (consumed on a 100 g basis) (Food and Drug Administration, 2014). Iron and zinc are found in low doses in cereals and tubers, which are a staple diet in many developing nations, especially in Africa, and therefore insects can be used to compliment these low iron and zinc diets. Meat is typically considered to be a good source of bioavailable iron and zinc (FAO & WHO, 2005), however, the insects in this study had superior iron and zinc contents to beef, pork and spinach (Fig. 3.4a & b).

a)



b)

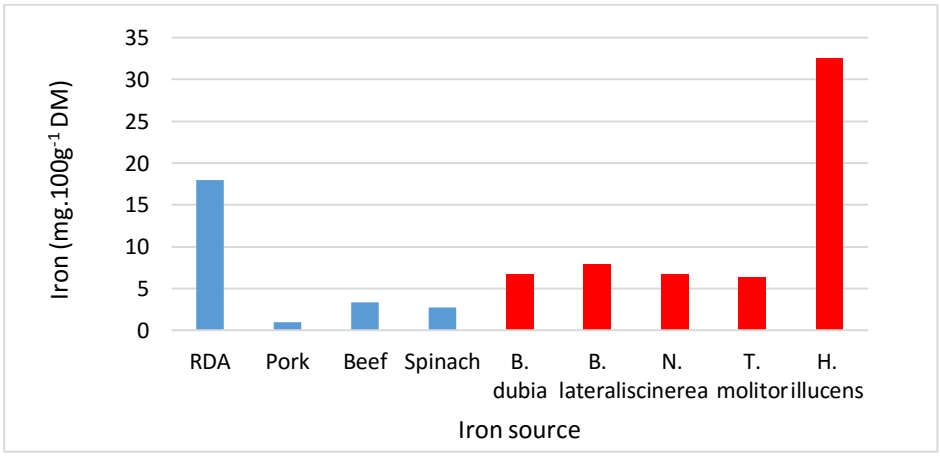


Figure 3.4 Iron (a) and zinc (b) content of five edible insect species (*Tenebrio molitor*, *Blatta lateralis*, *Blaptica dubia*, *Hermetia illucens* and *Naupheta cinerea*) compared to beef, pork, spinach and RDA (adapted from USDA, 2016).

Table 5.2 Mineral values for five blanched and unblanched insect species (mg.100g^{-1})

	Phosphorus	Potassium	Calcium	Magnesium	Sodium	Iron	Copper	Zinc	Manganese	Boron	Aluminium
N. cinerea	940.00	1520.00	180.00	150.00	655.00	6.77	3.00	29.38	1.09	0.44	1.50
Blanch *N. cinerea*	950.00	1490.00	300.00	170.00	816.20	7.64	3.22	30.72	1.68	0.40	1.80
B. lateralis	970.00	2310.00	280.00	170.00	678.60	7.97	4.16	21.52	1.78	0.49	1.10
Blanch *B. lateralis*	980.00	2300.00	310.00	160.00	820.70	7.55	4.09	21.24	1.99	0.43	0.91
T. molitor	1120.00	1110.00	100.00	450.00	203.40	6.32	0.92	15.04	3.20	0.19	0.94
Blanch *T. molitor*	1110.00	1100.00	70.00	450.00	117.10	5.76	1.67	14.93	3.16	0.16	1.02
B. dubia	740.00	1150.00	460.00	150.00	718.40	6.79	2.43	34.28	2.99	0.30	1.06
Blanch *B. dubia*	740.00	1170.00	350.00	140.00	591.00	6.46	2.82	38.20	1.64	0.21	0.98
H. illucens	1230.00	1680.00	5390.00	500.00	243.60	32.48	2.65	32.16	32.80	1.14	11.80
Blanch *H. illucens*	1310.00	1700.00	5350.00	530.00	228.00	30.56	2.72	34.24	36.34	0.85	8.40

Overall, potassium and phosphorus were the most abundant minerals in all of the insects that were tested, which is consistent with results of previous studies (Bukkens & Paoletti, 2005; Omotoso & Adedire, 2007; Chakravorty *et al.,* 2014; Hopley, 2016). The blanched and unblanched *H. illucens* were abundant in all of the minerals tested with values that exceed the daily recommended intake (FAO & WHO, 2005).

There is some controversy surrounding the aluminium content of foods as it has been suggested to contribute to the onset of Alzheimer's diseases (Stahl *et al.,* 2011; Bondy, 2016). Aluminium is often consumed in food products, however, it does not contribute to dietary requirements due to its low bioavailability. Efforts have been made to set limits regarding aluminium intake, however, the results of the studies are not completely conclusive at this stage. In 2007 the weekly limit was changed from 7 $mg.kg^{-1}$ body weight per week to 1 $mg.kg^{-1}$ body weight per week (Codex Alimentarius Commission, 2011), and was changed once again in 2011 to 2 $mg.kg^{-1}$ body weight per week, which based on the average male adult is 140 mg of aluminium per week (FAO, 2013). The insects in this study contain aluminium concentration ranging from 0.94 $mg.100g^{-1}$ in blanched *B. lateralis* to 11.80 $mg.100g^{-1}$ in *H. illucens*, which are slightly higher than most food sources (Stahl *et al.,* 2011; Bondy, 2016), however, using the current limits as guidelines even the aluminium for the *H. illucens* falls well below the weekly limit.

3.4.2 Fatty Acid Profile

The fatty acid composition varied between the insect species (Table 3.3). In *T. molitor, B. dubia* and *B. lateralis*, the proportion of unsaturated fat acids (UFA) was higher than that of saturated fatty acids (SFA). However, *H. illucens* and *N. cinerea* contained higher proportions of SFA than UFA. Insects are typically high in monounsaturated fatty acids (MUFA), with C18:1n9c (oleic acid) being the main fatty acid in most insect species (Tzompa-Sosa *et al.,* 2014; Womeni *et al.,* 2009; Chakravorty *et al.,* 2014; Oonincx, 2015; Zielińska *et al.,* 2015). Oleic acid was also the most prominent fatty acid in most of the insects tested in this study, with values ranging from 11.64 % in blanched *H. illucens* to 46.23 % in *B. dubia* (Table 3.3).

The polyunsaturated fatty acids (PUFA) range from 3.33 % in blanched *H. illucens* to 15.12 % in blanched *B. lateralis*. The fatty acids that are of utmost importance in the human diet are C18:2n6c (linoleic acid) and C18:3n3 (α-linoleic acid), as they play an integral role in brain development and cannot be synthesised in the body (James *et al.,* 2000).

Table 3.3 Fatty acid profile of five edible insect species (% of total fatty acids)

	C10:0	C14.0	C15.0	C16:0	C18.0	C20.0	Total SFA	C16:1	C18:1 n9c	C18:1 n9t	C20:1	C22:1 n9	Total MUFA	*C18:2 n6c	**C18: 3n3	*C20:2 n6	Total PUFA	Total UFA
N. cinerea	TR	0.81	0.10	36.29	19.40	0.51	55.80	4.79	27.04	7.52	TR	4.79	39.30	4.75	0.10	0.10	4.90	44.16
Blanch *N. cinerea*	ND	0.56	0.03	31.84	19.96	0.54	51.32	3.50	34.08	4.43	0.02	3.50	41.93	6.41	0.12	0.22	6.75	48.67
B. lateralis	TR	1.70	0.13	33.03	22.59	1.11	56.67	0.91	25.57	6.85	TR	0.91	33.09	8.62	1.49	0.12	10.24	43.32
Blanch *B. lateralis*	0.04	0.53	0.05	22.83	20.07	1.10	42.71	1.19	36.26	4.67	0.06	1.19	42.16	13.92	0.90	0.27	15.12	57.28
T. molitor	0.01	3.99	0.12	22.15	12.85	0.37	38.89	1.28	34.71	13.12	0.05	1.28	49.10	11.82	0.16	TR	12.01	61.10
Blanch *T. molitor*	ND	5.48	0.11	26.94	14.68	0.41	46.22	1.02	36.43	6.71	TR	1.02	44.07	9.58	0.09	0.04	9.71	53.78
B. dubia	TR	0.74	ND	19.37	20.40	0.68	39.61	2.90	46.23	4.27	0.09	2.90	53.39	6.48	0.13	0.38	6.99	60.38
Blanch *B. dubia*	TR	0.72	ND	22.79	18.81	0.60	41.17	2.22	37.53	12.72	0.07	2.22	52.42	5.69	0.08	0.63	6.41	58.83
H. illucens	0.23	12.89	0.46	7.69	11.22	0.29	69.38	0.86	13.40	7.93	TR	0.86	23.34	6.58	0.37	0.09	7.28	30.62
Blanch *H. illucens*	0.10	17.79	0.75	16.80	14.06	0.39	77.46	1.28	11.64	5.77	TR	1.28	19.21	3.12	0.08	TR	3.33	22.54

*Omega 6, **Omega 3, TR=trace amounts, ND= not detected

Previous studies have suggested that insects are good sources of both essential fatty acids linoleic (omega-6) and α-linolenic (omega-3) acid (Bukkens, 1997; Tzompa-Sosa *et al.,* 2014; Zielińska *et al.,* 2015), and it has been suggested that insects could play an integral role in contributing to the intake of omega-3 and -6 fatty acids, especially in landlocked countries where the supply of fish products are low (Fontaneto *et al.,* 2011; van Huis *et al.,* 2013). Linoleic acid concentrations in this study ranged from 3.12 % in blanched *H. illucens* to 13.92 % in blanched *B. lateralis* (Table 3.3). The α-linolenic acids were found in low concentrations ranging from 0.09 % in *N. cinerea* to 1.49 % in *B. lateralis*. The α-linolenic acid concentrations in this study are slightly lower than previous findings for insect species (Chakravorty *et al.,* 2014; Tzompa-Sosa *et al.,* 2014; Zielińska *et al.,* 2015), which can be attributed to the diet, as it has been found to alter the fatty acid content of insect species (Oonincx & Dierenfeld, 2012; Tzompa-Sosa *et al.,* 2014).

When compared to conventional food sources, the UFA of the insects were similar to beef and egg yolk with slightly higher PUFA than that of beef (USDA, 2016), but less UFA than salmon and flax seeds (Fig. 3.5). The exception being *H. illucens,* which had the lowest UFA content overall. It is important to take the high UFA content of insects into consideration when processing, as they are prone to oxidative rancidity and this can affect the quality of consuming insects (Shahidi & Wanasundara, 2002; van Huis *et al.,* 2013).

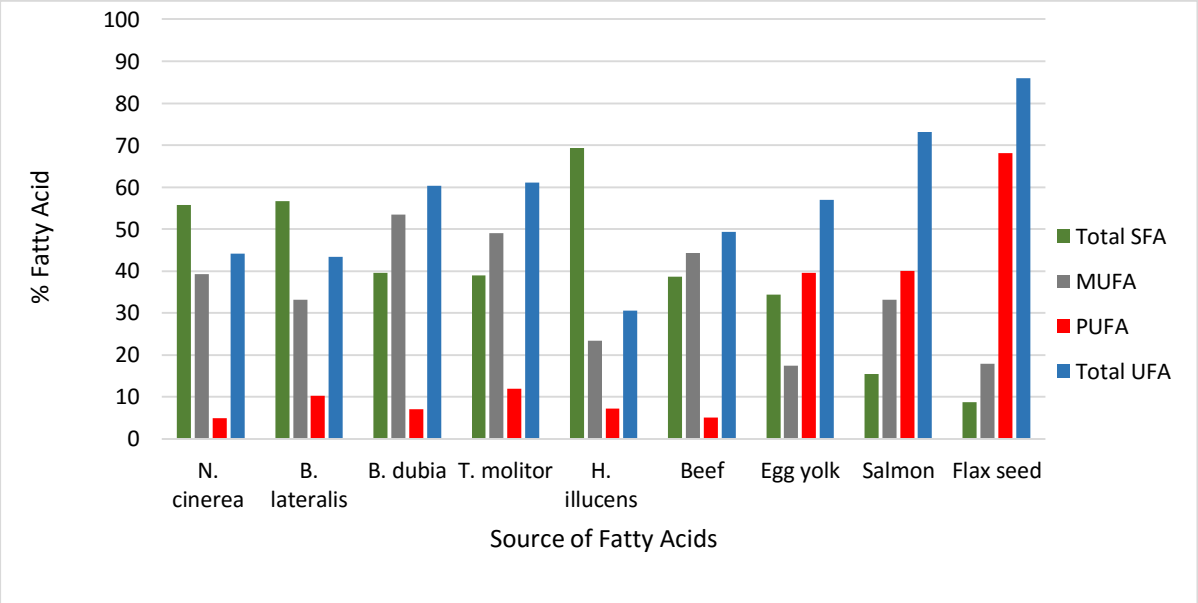


Figure 3.5 Saturated, monounsaturated, polyunsaturated and total fatty acid content of five edible insect species compared to various plant (flax seed) and animal (beef, egg yolk and salmon) sources (adapted from USDA, 2016).

The insects all contained relatively high SFA concentrations ranging from 38.97 % in *T. molitor* to 77.57 % in blanched *H. illucens*. These results are consistent with previous research where SFA compositions ranged from 27.1 % to 85.5 % across various insect species (Bukkens, 1997; Fontaneto *et al.,* 2011; Chakravorty *et al.,* 2014; Tzompa-Sosa *et al.,* 2014; Zielińska *et al.,* 2015). The SFA C16:0 (palmitic acid) and C18:0 (stearic acid) were the most abundant saturated fats in all of the insects tested, which has also been reported in other insect species (Bukkens, 1997; Fontaneto *et al.,* 2011; Chakravorty *et al.,* 2014; Zielińska *et al.,* 2015). *H. illucens* had high C14:0 (myristic acid) concentrations whilst all of other insect species had low C14:0 concentrations. Similarly, Oonincx (2015) found *H. illucens* to contain much higher myristic acid concentrations than other insect species. Evidence indicates that consuming foods high in SFA increase low density lipoproteins (LDL), leading to various cardiac health concerns. Myristic acid, found in relatively high concentrations in *H. illucens*, is the SFA that elevates LDL levels the most and can lead to a variety of cardiovascular diseases when eaten in large amounts (Zock *et al.,* 1994). Stearic acid on the other hand has been found to lower LDL levels and can help to reduce the incidence of cardiovascular disease (Bonanome & Grundy, 1988; Hunter *et al.,* 2010).

3.4.3 Amino Acid Profile

The roaches had higher amino acid contents than the *T. molitor* and *H. illucens* per 100 g of sample (Table 3.4), with the blanched *N. cinerea* having the highest amino acid values overall. This is as a result of the roaches having a higher protein content, and by default a higher amino acid content on a weight basis. When considering the concentration of each amino acid per gram of protein, the *T. molitor* and blanched *T. molitor* were found to have the highest concentration of each amino acid (Table 3.5). Leucine and valine were the most abundant essential amino acids in blanched *T. molitor*, with values of 63.4 $mg.g^{-1}$ protein and 54.1 $mg.g^{-1}$ protein, respectively. Similar results for *T. molitor* were found by Hopley (2016), Li *et al.* (2013) and Ghaly (2009). The results for *B. dubia* also compared well to results by Li *et al.* (2013). Methionine has been found to be the limiting amino acid in most insect species (Finke *et al.,* 1989; DeFoliart, 1992) and it was found to be the case in all the insect species tested in this study with methionine values ranging from 5.4 $mg.g^{-1}$ protein in the *B. lateralis* to 9.5 $mg.g^{-1}$ protein in the blanched *T. molitor* (Table 3.5). The amino acid, lysine, is of particular importance, as it is a limiting amino acid in many cereals, which forms the staple diet of many people worldwide, particularly in Africa. The daily lysine requirement for the average adult is 45 $mg.g^{-1}$ protein, with a protein intake of 54 g, and only the blanched and unblanched *T. molitor* met these requirements with lysine contents of 47.9 $mg.g^{-1}$ protein and 44.2 $mg.g^{-1}$ protein, respectively. The other insects in this study contained at least 60 % of the lysine requirements, all of which are much higher than the lysine values wheat, maize and rice (Fig.

3.6). The lysine content of the insects also compare well to soy protein, but are slightly less than beef and egg (USDA, 2016) (Fig. 3.6).

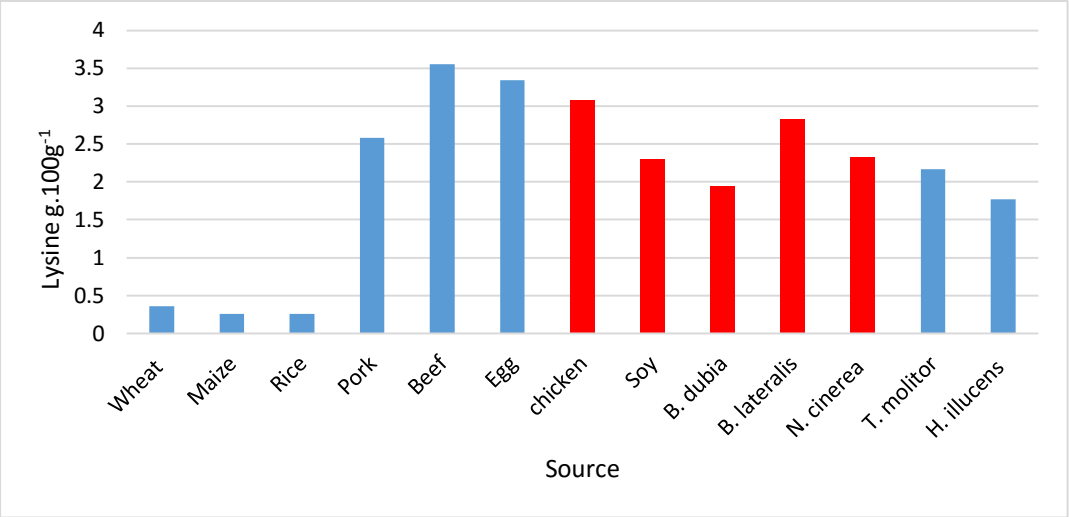


Figure 3.6 Lysine content of five edible insect species compared to some conventional plant and animal protein sources (adapted from USDA, 2016).

Table 3.4 Amino acid composition of five edible insect species in g.100g^{-1} sample (DM)

	*His	Ser	Arg	Gly	Asp	Glu	*Thr	Ala	Pro	Cys	*Lys	Tyr	*Met	*Val	*Ile	*Leu	*Phe
N. cinerea	1.32	2.06	2.66	3.55	3.53	5.01	1.80	3.83	2.76	0.11	2.32	3.26	0.58	3.04	1.82	3.09	1.75
Blanch *N. cinerea*	1.47	2.18	2.88	3.61	4.31	5.98	1.92	4.08	2.89	0.16	2.86	3.64	0.57	3.30	1.85	3.17	1.94
B. lateralis	1.36	2.00	2.86	3.11	3.71	5.85	1.86	3.75	2.52	0.14	2.83	2.90	0.54	2.93	1.73	2.91	1.63
Blanch *B. lateralis*	1.52	2.04	3.07	3.18	3.79	5.81	1.90	3.46	2.59	0.14	2.83	3.38	0.56	3.12	1.74	2.88	1.82
T. molitor	1.18	1.81	1.93	2.13	3.00	4.52	1.55	3.12	2.30	0.08	2.17	2.66	0.43	2.55	1.72	2.98	1.49
Blanch *T. molitor*	1.27	1.85	2.08	2.20	3.31	4.62	1.59	2.76	2.49	0.10	2.31	3.18	0.46	2.61	1.74	3.05	1.62
B. dubia	1.33	1.98	2.20	3.01	3.49	4.67	1.70	3.81	2.63	0.14	1.94	3.95	0.49	2.91	1.56	2.73	1.67
Blanch *B. dubia*	1.36	1.97	2.35	3.11	3.79	5.15	1.72	3.61	2.77	0.13	2.41	3.57	0.54	2.87	1.66	2.82	1.64
H. illucens	0.93	1.30	1.48	1.91	2.45	3.62	1.23	2.41	1.86	0.07	1.77	1.92	0.39	1.96	1.33	2.01	1.27
Blanch *H. illucens*	1.03	1.35	1.71	1.88	2.65	4.15	1.27	2.01	1.94	0.08	1.86	2.12	0.34	1.98	1.35	2.03	1.29

*Essential amino acids

Table 3.5 Essential amino acid composition of five edible insect species and daily requirement for adult in $mg.g^{-1}$ protein

	His	Thr	Lys	Phe	Met	Val	Ile	Leu
N. cinerea	17.13	23.33	30.06	22.69	7.52	39.31	23.51	39.98
Blanch *N. cinerea*	19.80	25.83	38.38	26.02	7.60	44.39	24.90	42.59
B. lateralis	13.36	18.35	27.84	16.02	5.35	28.83	17.06	28.65
Blanch *B. lateralis*	15.34	19.16	28.54	18.35	5.69	31.41	17.55	29.01
T. molitor	24.04	31.61	44.20	30.37	8.67	52.10	35.10	60.84
Blanch *T. molitor*	26.45	33.01	47.85	33.67	9.49	54.10	36.21	63.38
B. dubia	21.88	27.86	31.81	27.42	7.95	47.70	25.62	44.70
Blanch *B. dubia*	20.39	25.85	36.28	24.72	8.19	43.14	24.93	42.45
H. illucens	20.42	26.86	38.88	27.74	8.51	42.94	29.17	43.99
Blanch *H. illucens*	22.52	27.80	40.58	28.11	7.43	43.24	29.46	44.25
***Adult requirement**	15.00	23.00	45.00	38.00	22.00	39.00	30.00	59.00

* Joint WHO/FAO/UNU Expert Consultation (2007). Protein and Amino Acid Requirements in Human Nutrition. *World Health Organization technical report series, (*935), 1-265.

3.4.4 Microbiological Tests

Typically insects are eaten both raw and in a processed form (Banjo *et al.,* 2006a; Klunder *et al.,* 2012) and therefore it was important to evaluate the microbial load on the raw insect and post processing (blanching). Furthermore, the gut content of the insects can affect the microbiological quality of the insect (Klunder *et al.,* 2012), therefore the microbial load was evaluated after purging *T. molitor* and *H. illucens*. Both *T. molitor* and *H. illucens* are considered to have the biggest potential to be used in food and feed (EFSA Scientific Committee, 2015) and it is important to determine their their microbial safety. Currently there are no regulations for microbiological safety of edible insects, however, the Netherlands Food and Consumer Product Safety Authority have released a preliminary report with the micro-organisms relevant to insects and the limits of each (NVWA, 2014). The micro-organisms relevant to insects were based largely on the EU safety regulations for meat and seafood products (EU, 2005). The results of the microbial analysis are reported in Table 3.6, along with the preliminary guidelines suggested by the NVWA (2014).

Table 3.6 Microbial analysis of *T. molitor* and *H. illucens* at harvest, after being purged and blanched (cfu.g^{-1})

		T. molitor			***H. illucens***		
	NVWA Guidelines	**Harvest**	**Purged**	**Blanched**	**Harvest**	**Purged**	**Blanched**
Total viable count	*<5x10^5	>3x10^5	>3x10^5	<10	>3x10^5	>3x10^5	<10
Aerobic endospores	<10^5 (*B. cereus*)	<10	<10	<10	<100	<100	<10
Enterobacteriaceae	<10^2	>3x10^5	>3x10^5	<10	>3x10^5	>3x10^5	<10
***Salmonella* spp.**	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g
***Listeria* spp.**	<100	<5	<5	ND	ND	<5	ND

NVWA (2014). Advisory report on the risks associated with the consumption of mass-reared insects.https://www.nvwa.nl/documentennvwa/risicobeoordelingenvoedselveiligheid/bestand/2207475/gekweekte-insecten-ter-consumptie.

* EU (European Union) (2005). Commission regulation on microbiological criteria for foodstuffs (ec) no 2073/2005. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073.

The total viable count (TVC) and aerobic endospores were tested to determine the general quality of the insects and to give an indication of the general hygiene and potential

shelf-life (Jay *et al.,* 2005). The TVC on *T. molitor* and *H. illucens* was >3x10^5 cfu.g^{-1} at harvest and after purging (Table 3.6), which is within the criteria stipulated by the EU for meat and meat products (EU, 2005). Blanching the *T. molitor* and *H. illucens* reduced the levels to <10 cfu.g^{-1} (4.5 log reduction), which is regarded as safe for human consumption. The aerobic endospore count was <10 cfu.g^{-1} on all mealworm samples, which was lower than the amount of aerobic endospore formers on *H. illucens*. This can be attributed to their environment, as *T. molitor* were grown on cooked chicken feed, whereas the *H. illucens* were grown on vegetable matter. The aerobic endospore count on *H. illucens*, was <10^2 cfu.g^{-1} at harvest and after being purged, and after blanching it reduced to <10 cfu.g^{-1} (1 log reduction). The main aerobic endospore former of concern is *Bacillus cereus,* which produces enterotoxins that can withstand thermal treatments such as blanching. Guidelines advised by NVWA stipulate that there cannot be more than 10^5 cfu.g^{-1} of *B. cereus*, and in the case of the *T. molitor* and *H. illucens*, the levels of aerobic endospore formers are well below these advised limits (NVWA, 2014).

It has been suggested that, *Enterobacteriaceae,* need not be tested in insects, however, it was present in high amounts (>3x10^5 cfu.g^{-1}) at harvest and after purging in both *T. molitor* and *H. illucens*. Similar results were found on *Oryctes monocerus* (Banjo *et al.,* 2006a), *T. molitor* and *Acheta domesticus* (Klunder *et al.,* 2012), suggesting that *Enterobacteriaceae* is a concern when utilising insects in human food. Blanching reduced the *Enterobacteriaceae* levels to less than 10 cfu.g^{-1} (4.6 log reduction), which is below the guidelines set by the EU commission (EU, 2005). Common food pathogens, namely *Salmonella* and *L. monocytogenes,* were investigated as they are significant contributors to food borne illnesses across the world. Salmonella was not present in *T. molitor* and *H. illucens*. *Listeria monocytogenes* is not expected to be associated with insects (NVWA 2014), however, low levels (< 5 cfu.g^{-1}) of *Listeria* species were detected on purged *H. illucens* and harvested and purged *T. molitor.* Although the counts were low, and within the advised guidelines (NVWA, 2014), it is worth noting that *Listeria* species could be of concern in processed insects, and when testing insects for microbial safety.

These results show that the microbial load on both *T. molitor* and *H. illucens* are high, at harvest and after purging. The results from this study were consistent with studies on TVC, *Enterobacteriaceae*, *Salmonella* and *B. cereus* on various raw insect species in the Netherlands (NRVW, 2014), indicating that these micro-organisms are associated with many insect species, and should be considered routine micro-organisms to test for prior to consumption or processing. Blanching has been seen to reduce the microbial load to well below the recommended limits, and is considered the best heat treatment to decrease the microbial levels to within specifications, whereas roasting, and drying are not as effective in

reducing the microbial load (Klunder *et al.,* 2012; NVWA, 2014). As a result blanching is suggested prior to eating or further processing.

3.5 Conclusion

The results of this study compared well to previous research and indicated that insects are a good source of nutrients. *Tenebrio molitor* was found to have the highest fat content (35.8 g.100g^{-1}), whilst *B. lateralis* was found to have the highest protein values (101.5 g.100g^{-1}). The insects are considered a good source of energy and crude fibre, with energy values averaged at 24.1 MJ.kg^{-1}, and crude fibre values which ranged from 8.7 g.100g^{-1} in *T. molitor* to 19.1 g.100g^{-1} in *H. illucens*. All the insects were good sources of minerals, specifically iron and zinc, which exceeded daily requirements. *Hermetia illucens* contained the highest concentration of all the minerals, including calcium, which could easily meet the recommended daily requirement for an adult human. The amino acid profile of each insect species compared favourably to the daily requirement for the average adult, with the exception of methionine, which is considered to be the limiting amino acid in all of the insects tested. Oleic acid was the most prominent fatty acid in all of the insects tested, with values ranging from 11.6 % in blanched *H. illucens* to 46.2 % in the *B. dubia*, making insects potentially susceptible to oxidative rancidity. Linoleic acid was the highest PUFA and ranged from 3.3 % in the blanched *H. illucens* to 13.9 % in the blanched *B. lateralis*. The only omega-3 acid, α-linolenic acid, was found in low concentrations in the insect species. The insects all contained relatively high SFA concentrations with palmitic acid and stearic acid being the most abundant SFA.

In terms of microbial safety, *T. molitor* and *H. illucens* contained high TVC and high levels of *Enterobacteriaceae*. Blanching the *T. molitor* and *H. illucens* reduced the levels to <10 cfu.g^{-1} which was below the recommended load. The aerobic endospore count was low on *T. molitor* (<10 cfu.g^{-1}) and *H. illucens* (<10^{2} cfu.g^{-1}). Salmonella was not found on either insect. *Listeria* species could be a potential problem, as there was slight colony growth. These results can aid in forming microbial guidelines regarding insect consumption. Overall, blanching is suggested prior to eating or further processing insects, as it significantly reduces microbial levels to safe levels for human consumption and was also observed to aid in colour retention.

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Chapter 4

Exploratory investigation into the physical and functional properties of whole black soldier fly (*Hermetia illucens*) larvae.

4.1 Abstract

The physical characteristics and functional properties of whole black soldier fly larvae (BSFL) were investigated to determine its potential as a food ingredient. 'Pre-processing' treatments consisted of blanched and unblanched BSFL, and a fine and coarse mill of each. The physical tests performed determined the pH, water activity (a_w) and colour measurements of each treatment. The functionality tests determined both the water absorption and fat absorption capacity, emulsifying activity and stability, gelling properties, antioxidant activity and the lipid oxidation over 14 days of each treatment. The pH of BSFL ranged from 6.6 (unblanched) to 8.2 (blanched). Blanched BSFL was lighter in colour (L*=42, a*=2.5) than the unblanched BSFL (L*=35, a*=2.9). The BSFL had high a_w ranging from 0.985 to 0.991. Functional properties of the BSFL were established, indicating that BSFL had limited water absorption (104.2 % -105.2 %) and lipid absorption (104.8 % - 106.7 %) capacities. Gel formation only occurred in the unblanched samples, however, they were too weak and broke under pressure. Black soldier fly larvae also had adequate antioxidant activities ranging from 0.0043-0.018 Trolox µmol.mg^{-1}. Black soldier fly larvae was seen to have a poor emulsifying activity (1.67 % - 4.33 %) and stability (1.33 % - 6.67 %). It was observed that blanching reduced some functional properties of BSFL, such as the antioxidant capacity ($p<0.05$), water activity ($p<0.05$), and blanching resulted in no gel formation. Blanching did, however, have a positive effect on pH ($p<0.0001$), and prevented discoluration. Blanching had no effect on the other functional properties such as on the water absorption capacity, lipid absorption capacity, the emulsifying activity and stability, and the lipid oxidation of the BSFL. Time was seen to be the only factor that had a significant effect on the lipid oxidation ($p<0.05$). Overall, the results indicated that whole BSFL do have some functional properties, albeit limited.

Keywords: Black soldier fly larvae, insects as food, functional properties.

4.2 Introduction

Insects as a human food source has been gaining increasing attention worldwide due to their high nutritional composition, and especially their desirable protein content (Bukkens, 1997; Rumpold & Schlüter, 2013a; van Huis *et al.,* 2013; Yates-Doerr, 2015). The protein content of various insects are comparable to that of meat (Bukkens & Paoletti, 2005; Chakravorty *et al.,* 2014), making it a desirable alternative to meat, as well as, a reportedly more sustainable option (Oonincx *et al.,* 2010). Research has shown that Western consumers are more willing to consume insects when they are processed and disguised in a product, particularly in a meat-type product (Schösler *et al.,* 2012; Hartmann *et al.,* 2015; Tan *et al.,* 2015), therefore it is important to optimise processing procedures for insect products in order to introduce them into the Western food market. In order to determine the potential food application for insects, it is imperative to investigate the functional properties of the insect proteins, as well as, the insects in their entirety (Rumpold & Schlüter, 2013b). Functionality of an ingredient can be defined as 'any property of food or food ingredients, except it's nutritional ones, that influences its utilisation', such as viscosity, solubility, emulsification, gelling, foaming and water holding capacity (Hall, 1996). Most commonly the functionality of proteins contribute to creating and stabilising the characteristic structure of specific foods, for example, the characteristic foaming of egg whites; the curding of casein proteins in cheese and the emulsifying properties of meat proteins in sausages (Damodaran, 1994; Foegeding & Davis, 2011). The use of protein rich ingredients as additives usually depends on the functional characteristics it can impart to food (Adebowale *et al.,* 2005).

The functional properties of insect protein has received limited attention up to date, with the main focus being on the functional properties of insect flour (Omotoso, 2006; El Hassan *et al.,* 2008; Osasona & Olaofe, 2010; Womeni *et al.,* 2012; Assielou *et al.,* 2015). In 2013 Yi *et al.* studied the foaming and gelling properties of five different insect protein fractions. Insect protein was found to be a poor foaming agent, but it had the potential to be a gelling agent, specifically the house cricket (*Acheta domesticus*) which formed stable gels at a pH of 7 and a pH of 10 (Yi *et al.,* 2013). This particular study tested the proteins under ideal conditions, known as a 'model system', whereby the conditions of the system are known (Hall, 1996). Whilst this approach utilised standardised tests and provided quantitative values, the results may not predict the performance of the insect proteins in real food systems, therefore, there is merit in analysing the functionality of the insects in its whole form (Hall, 1996; Foegeding & Davis, 2011). The reason for this is that there are other food components, such as lipids, starches, and other constituents, that may either hinder or enhance the functionality

of the insect protein (Hall, 1996; Foegeding & Davis, 2011). These tests that investigate whole food ingredients are known as 'utility tests', and they test the proteins in conditions that mimic the food processing conditions (Hall, 1996).

Currently black soldier fly (*Hermetia illucens)* larvae is one of the main insects considered to have the biggest potential to be used in food and feed according to the European Union (EFSA Scientific Committee, 2015). The ultimate use of BSFL in food depends on its processing potential, therefore, the aim of this investigation was to analyse the characteristics and functional properties of whole BSFL to determine its potential as a functional food ingredient. Raw materials undergo various processing operations in order to produce commercial ingredients/products and some of these processing conditions can alter the functionality of a protein/ingredient (Hall, 1996). Heat treatments and particle size reduction are both known to affect the functionality of proteins (Hall, 1996; Perez-gago & Krochta, 2001; Wu, 2001) and were investigated in this study. This information would determine the desirable processing conditions and storage conditions of BSFL, and ultimately determine its suitability in food products. The functional properties of interest in this investigation were water and lipid absorption capacity, emulsifying activity and stability, gelling properties, antioxidant activity and lipid oxidation over time. Additionally, other characteristics of the BSFL were investigated, namely the pH, water activity and the colour.

4.3 Materials and Methods

4.3.1 Rearing and sample preparation

Black soldier fly larvae (BSFL) (1 kg) reared on chicken feed were randomly sampled at 10 days old from the Entomology Department at Wageningen University. The BSFL were sieved to remove the feed and then fasted for 24 h to clear their gastrointestinal tract. The BSFL were killed by freezing with liquid nitrogen, and stored at -20 °C until further analysis. Upon analysis, the BSFL were defrosted at 4 °C. Half of the BSF were blanched for 2 min at 98 °C and the other half remained unblanched to determine if blanching had an effect on both the physical and functional properties. Two sets of blanched and unblanched samples were homogenised separately in a waring blender on the slowest speed for two time variations to obtain a paste with fine milling size and a coarse milling size (Fig. 4.1). These four different treatments, namely blanched fine paste, blanched coarse paste, unblanched fine paste and unblanched coarse paste, were tested for physical and functional properties in triplicate to determine the effects of both blanching and particle size on the functional properties (water absorption capacity, lipid absorption capacity, emulsifying activity, emulsifying stability, gelling properties, lipid oxidation and antioxidant activity) of BSFL.

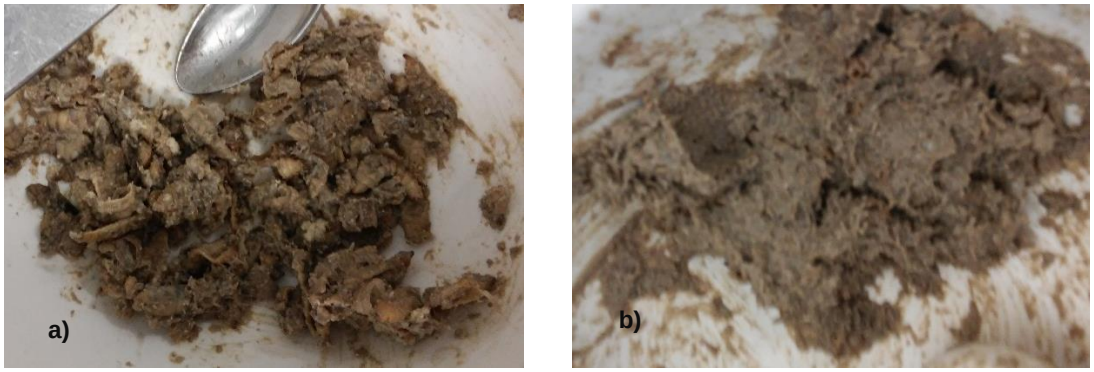

Figure 4.1 Coarse black soldier fly larvae paste (a), and fine black soldier fly larvae paste (b).

4.3.2 Characteristics of paste

The pH was determined using a direct pH method, whereby 10 g of sample was mixed together with 10 mL of distilled water and the pH was read using a glass electrode (pHenomenal® LS 221, VWR, Hunter Boulevard, Leicestershire LE17 4XN, UK) (Korkeala *et al.,* 1986). The colour (CIEL*a*b*) of each sample was analysed in triplicate using the HunterLab Colorflex Spectrophotometer (ELSCOLAB, Niels Bohrweg 155, 3542 CA Utrecht, Nederland). L* being an indication of the lightness, a* an indication of the red-green range and b* an indication of the yellow-blue range. The CIEL*a*b* colour meter was standardised using the white and black standard, and the green standard was used to ensure that the calibration was accurate. Each sample (5 g) was placed in a cuvette and three colour measurements were recorded per sample. Water activity (a_w) was measured using the Novasina water activity meter (Neuheimstrasse12CH-8853 Lachen Switzerland), on setting 2 and 1, at a temperature of 25 °C. Each sample (6 g) was placed in a cuvette and placed into the water activity meter until a stable reading was displayed and recorded.

4.3.3 Functional properties

4.3.2.1 Water absorption and lipid absorption capacity

Water absorption capacity (WAC) was determined using the method as described by Sathe and Salunkhe (1981) with slight modifications. The sample (1 g) was placed together with 10 ml of MilliQ water (ultra-purified water) into a pre-weighed 15 ml centrifuge tube and mixed at 1 000 rpm for 5 min using the T 10 Ultra-Turrax homogeniser. The sample was then centrifuged at 2 060 x g for 10 min. The water that was not retained was discarded and the centrifuge tube with sample retaining water was weighed. The WAC was calculated as the percentage of water retained by 1 g of sample. Lipid absorption capacity (LAC) was determined using the method described by Sosulski (1976), where 0.3 g of sample was placed together with 3 ml of sunflower oil into a pre-weighed 15 ml graduated centrifuge tube and mixed at 1 000 rpm for 5 min using the T 10 Ultra-Turrax homogeniser. The solution was then centrifuged at 2 060 x g for 30 min. The oil that was not retained was discarded and the cylinder

with the sample and retained oil was weighed. The LAC was calculated as the percentage of oil retained by 1 g of sample.

4.3.2.2 Emulsifying activity and stability

The emulsifying activity and emulsion stability were determined using the method described by Yasumatsu *et al.* (1972). Each sample (7 g) was homogenised with 100 ml of water and 100 ml of sunflower oil using an ultraturrax at 11 000 rpm for 1 min. The homogenised sample was then dispensed into 50 ml centrifuge tubes, and centrifuged at 1 300 x g for 5 min. The emulsifying activity was then determined using equation 4.1 (Yasumatsu *et al.,* 1972). The height in the tube was determined in millimetres according to the markings on the tube.

$$\boldsymbol{Emulsifying\ activity} = \frac{\boldsymbol{height\ of\ emulsified\ layer}}{\boldsymbol{total\ height\ of\ liquid\ in\ tube}}\boldsymbol{x100} \qquad \textbf{(Equation 4.5)}$$

Emulsion stability was determined by preparing the emulsion in the same way as described above, however, the homogenised sample was heated for 30 min at 80 °C prior to centrifugation. Emulsion stability was calculated using equation 4.2 (Yasumatsu *et al.,* 1972).

$$\boldsymbol{Emulsion\ stability} = \frac{\boldsymbol{height\ of\ remaining\ emulsified\ layer}}{\boldsymbol{total\ height\ of\ liquid\ in\ tube}}\boldsymbol{x100} \qquad \textbf{(Equation 4.6)}$$

4.3.2.3 Gelling properties

Gel formation

Gel formation was tested using the same method described by Yi *et al.* (2013). Due to the nature of the test, the blanched and unblanched BSFL were dried at 60 °C for 48 h and milled into a fine powder prior to analysis. Each sample was dissolved at pH 3, pH 7 and pH 10 at concentrations of 40 %, 50 % & 60 % $w.v^{-1}$. The pH of the solution was obtained by adding HCl or NaCl to MilliQ water until the correct pH was reached. The pH was read using a glass electrode (pHenomenal® LS 221, VWR, Hunter Boulevard, Leicestershire LE17 4XN, UK). The effect of ionic strength was also tested by repeating all the samples with a 1M sodium chloride inclusion. The solutions were vortexed for 30 min and 1 ml was placed in a 2 ml Eppendorf tube and heated in a water bath at 85 °C for 30 min. Once removed from the water bath, samples were cooled at 4 °C for 10 min. After 10 min gel formation was assessed visually, by turning the Eppendorf tubes upside down. Those where the liquid did not move were considered a gel (Yi *et al.,* 2013).

Gel strength

Gel strength was quantified using the compression test with a TA.XT *plus* Texture Analyser (Stable Micro Systems, LTD, Surrey, UK). The Eppendorf tubes were placed in an Eppendorf tube stand and placed on the platform of the texture analyser. A pin head with a 2 mm diameter was attached to a 500 g load cell and the pin penetrated the sample once at a speed of 0.1 $mm.s^{-1}$ until midway into the Eppendorf tube to determine the strength of the gel.

4.3.2.4 Antioxidant activity

Total antioxidant activity was determined using the Quencher method as described by Serpen *et al.* (2012). Antioxidant activity was measured spectrophotometrically according to it's free-radical scavenging capacity with the radical cation DPPH (2,2-diphenyl-1-picrylhydrazyl) which has an absorption band at 525 nm. In the presence of an antioxidant, DPPH became reduced and disappeared. Due to the nature of the test the blanched and unblanched BSFL were dried at 60 °C for 48 h and milled into a fine powder prior to analysis. In order to compare the antioxidant activity, a dried mango sample was used as a standard (Gökmen *et al.,* 2009). The samples were then diluted with powdered cellulose in a ratio of 1:1, and then 10 mg, 20 mg and 40 mg of this mixture were added to 5 ml of 0.1 mM DPPH in Eppendorf tubes, covered in foil, shaken and incubated at room temperature for 100 min (Gökmen *et al.,* 2009). A 20 $mg.ml^{-1}$ stock solution of Trolox was diluted and a 5 point calibration curve was made with concentrations ranging from 3.75 $mg.ml^{-1}$ to 0.50 $mg.ml^{-1}$. Ten µl of these calibration samples were added to 10 mg of cellulose in a 5 ml Eppendorf tube with 5 ml 0.1 mM DPPH. The Eppendorf tubes were then covered in foil, shaken and incubated at room temperature for 100 min (Gökmen *et al.,* 2009). Duplicate blanks comprised of cellulose and DPPH, were also covered in foil, shaken and incubated at room temperature for 100 min. The linear regression of the graphs were calculated and the equations below were used to obtain the concentration of Trolox measured in µmol of Trolox per mg of dried sample (Equation 4.3, Equation 4.4 & Equation 4.5).

$$\mathbf{50\ \%\ DPPH\ inhibition\ value} = \frac{CR50 - c}{R} \qquad \textbf{(Equation 4.7)}$$

Where:

CR50 = average of blank ($\lambda 525nm$)/n, where n is number of blanks

R = slope of calibration curve

C = y intercept

$$\boldsymbol{CR50\ sample\ mg} = \frac{\boldsymbol{CR50-c}}{\boldsymbol{M}} \qquad \textbf{(Equation 4.8)}$$

Where:

CR50 = average of blank ($\boldsymbol{\lambda 525nm}$)/n (where n=number of blanks)

M = slope of sample linear regression curve

C = y intercept of sample linear regression

$$\boldsymbol{trolox}\ \mu\boldsymbol{mol.mg^{-1}} = \frac{\boldsymbol{50\ \%\ DPPH\ inhibition\ value}}{\boldsymbol{CR50\ of\ sample}}\boldsymbol{x1000} \qquad \textbf{(Equation 4.9)}$$

4.3.2.5 Lipid oxidation

Lipid oxidation was tested over a 14 day period to determine the level of lipid oxidation over time. Lipid oxidation was measured by the production of the secondary oxidation product, malondialdehyde using the spectrophotometric Thiobarbuturic acid method (TBA-RS) (de las Heras *et al.,* 2003). The samples (6 g) were homogenised with 50 ml of milliQ water with an ultraturrax at a speed of 11 000 rpm for 1 min. The samples where then centrifuged at 3 000 rpm for 5 min before filtering the samples through Whatman $595^{1/2}$ filter paper (Schleicher en Schuel). In a klimax tube 5 ml of the filtrate and 5 ml of 15 % w.w^{-1} Trichloroacetic acid (TCA) was vortexed and filtered once again through 0.45 µm 25 mm cellulose acetate membrane filter. The filtrate (2 ml) was added to 2 ml of Thiobarbituric acid (TBA), vortexed and incubated at 100 °C for 35 min along with a dilution series of TBA concentrations. The absorbance of the samples and the TBA dilution series was measured at 532 nm against a blank of TBA and TCA. The TBA dilution series was used to construct a calibration curve, whereby Equation 4.6 was used to calculate the concentration of malondialdehyde in each sample. The samples were kept in the dark, at refrigerated temperatures (4 °C), in an airtight container throughout the 14 d period (Day 0, 3, 7 &14).

$$\boldsymbol{mg\ maliodialdehyde/kg} = \frac{\boldsymbol{(\lambda 532nm)x\frac{C}{G}}}{\boldsymbol{R}} \qquad \textbf{(Equation 4.10)}$$

Where:

R = slope of calibration curve

C = 50/2 (volume extract 50 ml; volume of test sample 2 ml)

G = mass of sample

4.3.4 Statistical analysis

All data was analysed using SPSS software Analysis of Variance (ANOVA) and Univariate Analysis of Variance for treatment and milling combination. Independent T-test for emulsions and anti-oxidant.

4.4 Results and Discussion

4.4.1 Characteristics of paste

The pH of the BSFL ranged from 6.59 in the unblanched fine samples to 8.24 in the blanched coarse sample (Table 4.1). Blanching the BSFL seemed to result in a higher ($p<0.0001$) pH than that of the unblanched BSFL. It was speculated that blanching denatured the proteins, preventing the enzymatic reactions that caused the decrease in pH in the unblanched BSFL. The enzymatic reactions occur rapidly when in contact with air, and it has been seen visually with colour retention in this study (within 2 mins), and previously by Klunder *et al.* (2009).

Table 4.6 Average of the results and standard deviation of physical characteristics of blanched and unblanched BSFL (N=3)

	BBSF fine	**BBSF coarse**	**BSF fine**	**BSF coarse**
pH	8.17 ± 0.06	8.24 ± 0.03	6.59 ± 0.06	6.68 ± 0.02
Colour L*	41.76 ± 0.01	42.65 ± 0.06	35.50 ± 0.24	34.62 ± 0.21
Colour a*	2.36 ± 0.01	2.85 ± 0.06	2.81 ± 0.03	3.08 ± 0.70
Colour b*	12.27 ± 0.01	13.61 ± 0.03	13.62 ± 0.21	13.71 ± 0.02
a_w	0.987 ± 0.00	0.985 ± 0.00	0.988 ± 0.00	0.991 ± 0.00

BBSF-Blanched BSFL; BSF- Unblanched BSFL

The L* values of the BBSFL samples were seen to be higher ($p<0.05$) than that of the unblanched BSFL samples, indicating that blanching prevented discolouration of the BSFL. Blanching prevented discolouration of raw crickets and grasshoppers, by denaturing the enzymes that caused colour deterioration (Klunder *et al.,* 2012). L* values are used to indicate levels of enzymatic browning in vegetables and fruit, with low L* values indicating enzymatic browning (Reis *et al.,* 2008). The results from this study indicate that L* values can also be used to determine enzymatic browning in BSFL, as blanching the BSFL resulted in a visually lighter colour, with higher L* values than that of the unblanched BSFL (Fig. 4.2).

Figure 4.2 Showing the colour difference between unblanched BSFL (left) and blanched BSFL (right).

Enzymatic browning requires the presence of oxygen (Reis *et al.,* 2008), therefore the finer milled paste, which has a greater surface area exposed to oxygen, was expected to have a darker colour than that of the coarse milled BSFL (Table 4.1). The redness scale (a*) has also been used to indicate levels of enzymatic browning, with an increase in a* values indicating enzymatic browning (Reis *et al.,* 2008). The unblanched BSFL had higher ($p<0.0001$) a* values than that of the blanched BSFL (Table 4.1), also as a result of the blanching denaturing the enzymes that cause enzymatic browning.

The a_w of the BSFL ranged from 0.985 in the blanched BSFL coarse sample to 0.991 in the unblanched BSFL coarse sample (Table 4.1). Blanched BSFL has slightly lower ($p<0.05$) a_w values than the unblanched BSFL. Similar results were observed in blanched potatoes, where blanching reduced the a_w of potatoes (Reis *et al.,* 2008).

4.4.2 Functional Properties

4.4.2.1 Water absorption and lipid absorption capacity

The water absorption capacity (WAC) of BSFL ranged from 104.20 % in the fine unblanched BSFL to 105.23 % in the coarse unblanched BSFL (Table 4.2). Currently the results can only be compared to the WAC of dried, milled insects, therefore there is expected to be differences between the results. The results of this study are lower than previous findings, where dried *Oryctes owariensis* (Raphia weevil) larvae were found to have a high WAC of 220 % (Assielou *et al.,* 2015) and dried *Cirina forda* (Emperor moth) larvae which had high WAC ranging from 248 % to 300 % (Omotoso & Adedire, 2007; Osasona & Olaofe, 2010). Research has shown that the moisture content of an ingredient directly influences the WAC (Barbut, 1999) and it is expected that the BSFL larvae would have higher water absorption capacities in a dried form, much like that of the dried insects previously investigated (Omotoso & Adedire, 2007; Osasona & Olaofe, 2010; Assielou *et al.,* 2015).

Table 4.7 Results and standard deviations of the water absorption and lipid absorption capacity of BSFL, expressed as percentages of BSFL (N=3).

Test	BBSF fine	BBSF coarse	BSF fine	BSF coarse
WAC (%)	104.51 ± 0.24	104.23 ± 0.79	104.20 ± 1.83	105.23 ± 2.48
LAC (%)	106.39 ± 0.61	106.03 ± 2.02	104.76 ± 1.25	106.69 ± 2.01

The lipid absorption capacity (LAC) of BSFL ranged from 104.76 % in the fine unblanched BSFL to 106.69 % in the coarse unblanched BSFL (Table 4.2). The LAC of BSFL was substantially less than that of dried *O. owariensis* larvae which had a high LAC of 178 % (Assielou *et al.,* 2015) and dried *C. forda* larvae which had high LAC ranging from 265 % to 358 % (Omotoso & Adedire, 2007; Osasona & Olaofe, 2010). Similar to the WAC, it is expected that dried BSFL would have higher LAC than the wet BSFL.

The ability of proteins to absorb and retain both water and oil contributes greatly to the overall taste, texture and mouthfeel of food products (Adebowale *et al.,* 2005). From these results alone BSFL are not considered to have particularly good water and lipid absorption capacities when comparing them to dried insects (Omotoso, 2006; El Hassan *et al.,* 2008; Osasona & Olaofe, 2010; Womeni *et al.,* 2012; Assielou *et al.,* 2015) and other commercial ingredients (Aremu *et al.,* 2008; Chandra & Samsher, 2013). Further research into the WAC and LAC of dried BSFL are expected to yield higher absorption capacities.

4.4.2.2 Emulsifying activity and stability

The emulsifying activity (EA) of BSFL was poor ranging from 3.33 % in the fine blanched BSFL to 4.33 % in the unblanched coarse BSFL (Fig. 4.3). The 1 M salt inclusion seemed to decrease the emulsifying activity slightly, with values ranging from 1.67 % in the blanched BSFL coarse to 4.00 % in the unblanched BSFL fine (Fig. 4.3). Salt has been found to decrease the emulsifying activity in many food ingredients (Khalid *et al.,* 2003; Ragab *et al.,* 2004) due to the effect on the zeta potential, which affects the particle absorption at the oil-water interface (Yang *et al.,* 2006).

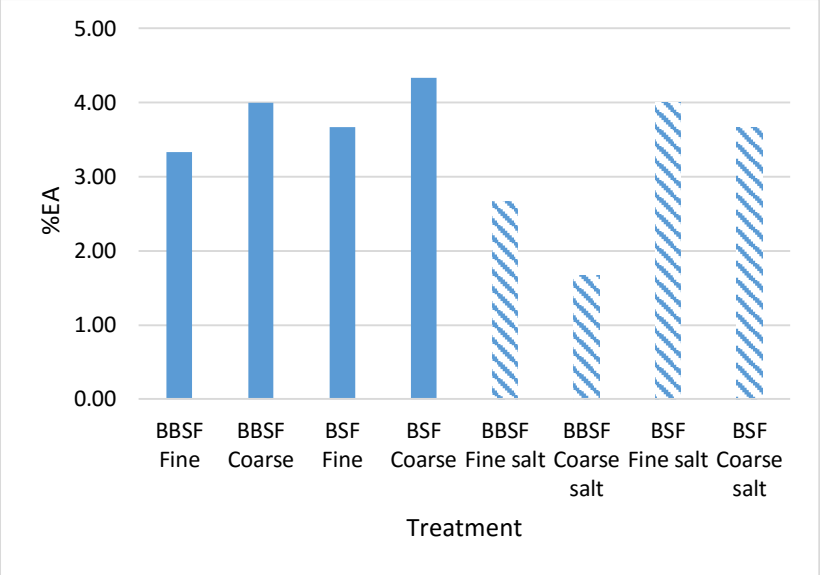

Figure 4.32 The emulsifying activity (EA) of BSFL measured as a percentage of the total volume that formed an emulsion.

There are no standard measurements for assessing emulsion formation and stability, therefore it is not always possible to draw clear comparisons (Patel & Fry, 1987; Hall, 1996). Due to the turbid nature and dark colour of the samples, the turbidimetric technique proposed by Kinsella and Pearce (1978) which measures emulsification spectrophometrically based on change in turbidity was not suitable for this investigation. The technique used by Yatsumatsu *et al.* (1972) was found to be more suitable for the samples in this investigation, as it involved measuring the emulsified layer against the total height of the solution in a tube. When comparing the values in this study to dried insect powder, the EA values of BSFL are much lower. Dried *O. owariensis* larvae has been found to have an EA of 29.7 % (Assielou *et al.,* 2015) and dried *C. forda* larvae had EA of 36.67 % (Omotoso & Adedire, 2007). When comparing BSFL to ingredients that are commonly used as emulsifiers, BSFL in its whole form is considered to have relatively poor emulsifying activity (Yasumatsu *et al.,* 1972; Marcone & Kakuda, 1999; Wu, 2001). Amaranth globulin isolate had EA ranging from 50.2 % to 79.3 %, soybean globulin isolate had EA ranging from 40 % to 53.7 % (Marcone & Kakuda, 1999) and corn gluten meal had EA of 56.2 % (Wu, 2001). In comparison to these emulsifiers, BSFL at the highest EA of 6.67 %, would not be considered a good or stable emulsifier in food applications. This investigation only considered the effects of blanching and salt inclusion as factors influencing the EA of BSFL, however, future investigations should consider particle size, pH, and isolating the proteins in order to optimise the EA of BSFL (Pearce & Kinsella, 1978; Marcone & Kakuda, 1999; Wu, 2001). Furthermore, it is expected that dried BSFL would have better EA and emulsion stability (ES).

Thermodynamically, emulsions are not considered stable states, however, those that are considered functionally stable undergo the process of oil and water separation much slower than those considered unstable (Pearce & Kinsella, 1978). The ES of the BSFL ranged from 1.33 % in the blanched fine BSFL to 4.33 % in both unblanched samples (Fig. 4.4)

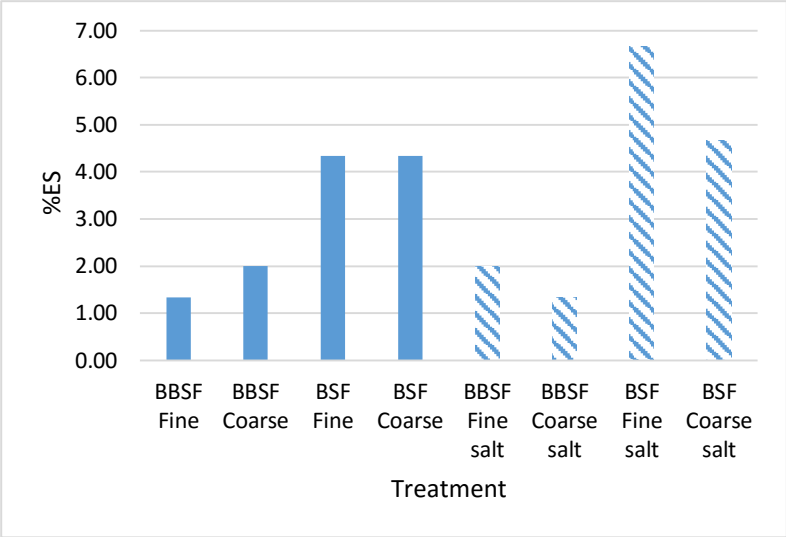

Figure 4.43 The emulsifying stability (ES) of BSFL measured as a percentage of the total volume that remained an emulsion.

4.4.2.3 Gelling properties

Gel formation

Sample concentration (% w.v^{-1}), pH and salt concentration were the three variables tested in this investigation, as they are factors that typically affect gel formation (Hall, 1996). Upon visual observation it was seen that there was no gel formation amongst the blanched samples (Table 4.3. When the tubes were inverted the liquid immediately fell to the bottom, indicating that no gel was formed. These results were expected, as blanching is known to denature proteins reducing many of the proteins functions (Boye & Harwalkar, 1997). With regard to the unblanched samples, all of the solutions formed a gel with the exception of those marked '-' in Table 4.3.

Gel strength

The samples that formed gels were tested for gel strength, but as they were too weak to retain shape upon compression from the texture analyser, no conclusive data was obtained from the compression test. Based on the visual observations (Fig. 4.5, Fig. 4.6 & Fig. 4.7) it was speculated that both pH and ionic strength may have had a slight effect on the gel formation of BSFL. It was observed that at pH 3 there was no gel formation at both the 40 % and 60 % concentrations. Similar findings were documented by Li *et al.* (2013), where five different

insect protein fractions did not form a gel at pH 3 at both low and high protein concentrations. Additionally the visual observations suggest that the addition of salt may hinder the formation of the gels (Fig. 4.5, Fig. 4.6 & Fig. 4.7).

Table 4.3 Results of BSFL gel formation under various pH and ionic strength conditions.

			Blanched	Unblanched
% of sample	pH	Ionic strength	-	+
40 %	control	control	-	+
	control	1M	-	+
	3	control	-	+
	3	1M	-	-
	10	control	-	+
	10	1M	-	-
50 %	control	control	-	+
	control	1M	-	+
	3	control	-	+
	3	1M	-	+
	10	control	-	+
	10	1M	-	-
60 %	control	control	-	+
	control	1M	-	+
	3	control	-	+
	3	1M	-	-
	10	control	-	+
	10	1M	-	+

+ = gel formation; - = no gel formation

With regard to the 40 % $w.v^{-1}$, all the solutions formed a gel (Fig. 4.5), with the exceptions of pH 3 with 1 M salt, and pH 10 with 1M salt. Additionally, judging from the visual observation, the pH 3 and 10 without the salt inclusion do not form a stable gel (Fig. 4.5)

Figure 4.5 Gel formation of 40 % w.v^{-1} unblanched BSFL gels from pH 10 sample (left) to control sample (right).

With regard to the 50 % w.v^{-1}, all formed gels, except pH 10 with the 1 M salt inclusion (Fig. 4.6).

Figure 4.4 Gel formation of 50 % w.v^{-1} unblanched BSFL gels from pH 10 sample (left) to control sample (right).

At pH 3 with the 1 M salt inclusion, the 60 % w.v^{-1} sample did not form a stable gel upon inversion (Fig. 4.7).

Figure 4.7 Gel formation of 60 % w.v^{-1} unblanched BSFL gels from pH 10 sample (left) to control sample (right).

A recommendation for future investigations would be to do rheological tests on the gels in an effort to obtain some quantitative data. Rheological testing requires a larger volume of sample per test, and this investigation did not have a large enough volume of sample to perform the rheological tests.

4.4.2.4 Antioxidant activity

In this investigation, the antioxidant activity was measured in Trolox equivalents, which quantified the ability of BSFL to 'quench' the radical cation DPPH in comparison to Trolox, a

vitamin E analogue, which has a strong antioxidant capacity (Pellegrini *et al.,* 2003). Due to the nature of the test, dried fine particles were required, and therefore milling size was not taken in account in this particular test. The results showed that there was a difference between blanched and unblanched treatments (Fig. 4.8). Blanching the samples was seen to reduce ($p<0.05$) the antioxidant capacity of the BSFL samples. This was expected, as blanching has been seen to cause losses in total antioxidant activity in various other food products, such as leafy green vegetables (Oboh, 2005), peanut skins extracts (Yu *et al.,* 2005) and Brassica vegetables (Yu *et al.,* 2005; Podsędek, 2007). The unblanched BSFL have adequate antioxidant activity, however, the mango standard, which is well-known for being rich in anti-oxidants (Kaur & Kapoor, 2001), was found to have 2.2 times the antioxidant capacity of unblanched BSFL.

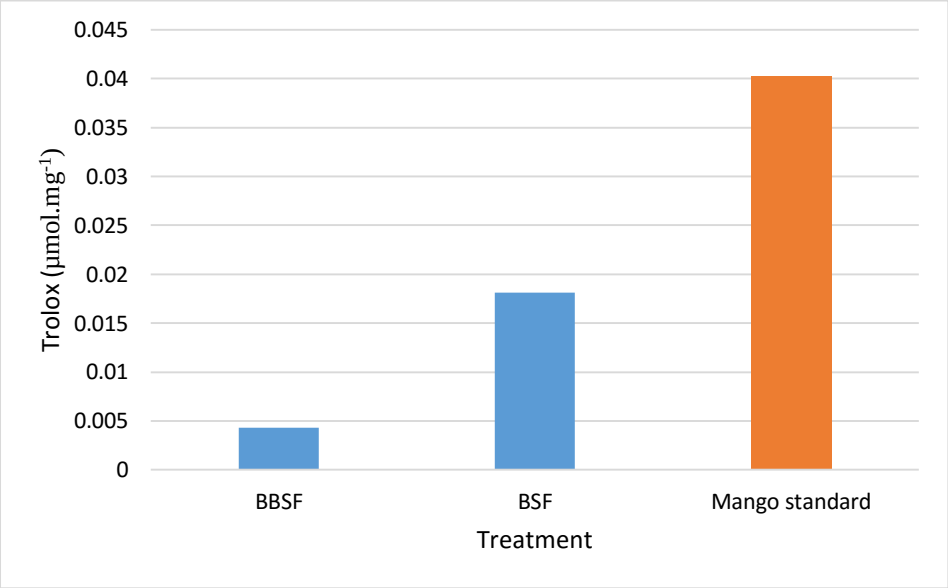

Figure 4.8 Antioxidant activity of blanched and unblanched treatments, measured in Trolox concentration.

Oxidation can have many negative effects on foods, causing chemical spoilage resulting in deterioration of colour, flavour, food safety and nutritional quality. Antioxidants are substances which, at low concentrations, significantly inhibit or prevent the oxidation of a substrate (Antolovich *et al.,* 2002). When analysing the antioxidant capacity of an isolated compound, the values obtained for the antioxidant activity does not reflect the total antioxidant capacity of the food ingredient, as it does not take into account the synergic and redox reactions that take place amongst the various molecules in the food ingredient (Pellegrini *et al.,* 2003). Antioxidants are used in food manufacturing for its functionality in the food product,

as they act as a defence mechanisms against oxidation, preventing various forms of oxidative spoilage (Antolovich *et al.,* 2002).

4.4.2.5 Lipid oxidation

Investigations into the composition of edible insects have shown that the majority of edible insects contain high concentrations of unsaturated fatty acids (Bukkens & Paoletti, 2005; Womeni *et al.,* 2009; Chakravorty *et al.,* 2014; Tzompa-Sosa *et al.,* 2014), which increases the risks of lipid oxidation and rancidity occurring, especially during processing (Finley & Otterburn, 1993). Malondialdehyde is one of the products of oxidative stress, and is commonly used to indicate and quantify oxidative damage and rancidity in foods (Botsoglou *et al.,* 1994; Bergamo *et al.,* 1998).

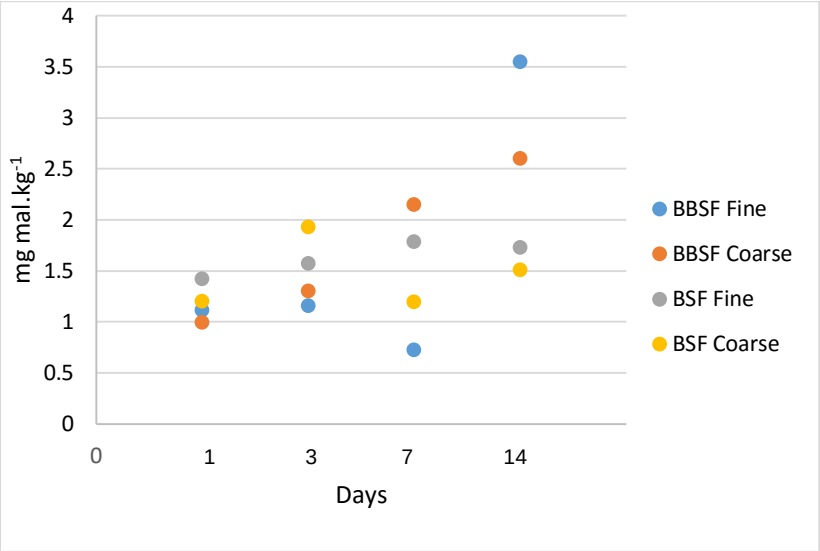

Figure 4.9 Lipid oxidation of BSFL in refrigerated conditions measured over 14 days (mg malondialdehyde.kg^{-1}).

The lipid oxidation results show that there were no differences in malondialdehyde concentration between the blanched and unblanched treatments; as well as, between the fine and coarse mill (Fig. 4.9). There was an increase in lipid oxidation over time, however, the malondialdehyde concentration did not increase much within the first 7 days. The malondialdehyde concentration increased ($p<0.05$) between day 7 and 14, indicating that lipid oxidation mainly took place after 7 days of refrigeration (Fig. 4.9). It is difficult to compare these results to previous studies, as it has not been done on BSFL before, however, the malondialdehyde concentration of the BSFL over the first 7 days was similar to the malondialdehyde concentration of chicken breast over 7 days of refrigerated storage (Sampaio *et al.,* 2012). Similarly, the malondialdehyde concentration range of BSFL in the first

7 days is similar to minced meat (Jung *et al.,* 2016). As a result, it is speculated that the protocols used to prevent and control lipid oxidation in meat can also potentially be used in BSFL. Validation studies would need to be done to confirm this.

4.5 Conclusions

The results from this investigation indicate that BSFL do have some functional properties, and can therefore be used in certain applications, however, the extent of the functionality of BSFL is somewhat limited. For example, BSFL can be used to aid in water and lipid absorption, gelling and antioxidant activity, however, it would need additional ingredients in order to perform the functions fully as it does not compare well with commercial functional ingredients. Blanching showed inconsistent results by reducing some of the functional properties, as well as, having no effect on other functional properties such as water and lipid absorption capacity. Additionally blanching had a positive effect on the colour retention of the BSFL, and it has previously been seen to significantly reduce microbial load (Klunder *et al.,* 2012), therefore strong considerations would need to be made regarding the final product and functional properties needed from the BSFL before deciding to use blanching as pre-processing treatment. If blanching affects the functions that are needed in processing, efforts would have to be made to find alternative ways to decrease the microbial load to a satisfactory level, as well as, prevent enzymatic browning whilst still maintaining the functional properties. Milling size does not affect the functionality of BSFL, it was only seen to have an effect on the colour with regard to the lightness and the yellowness of the BSFL. Therefore, as a pre-process, various milling sizes can be used without needing to consider how it would affect the functioning of the ingredient. Further research could include various processing conditions, such as pH, and drying could be used to try optimise the functional properties.

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Chapter 5

Use of black soldier fly (*Hermetia illucens*) larvae in the production of a vienna-type sausage.

4.1 Abstract

The aim of this study was to determine whether black soldier fly larvae (BSFL) could be processed into a vienna-type sausage. Four different treatments were investigated to obtain a BSFL vienna-type sausage that was most comparable to that of a commercial pork vienna sausage recipe. Treatments consisted of a pork vienna (control) and three different BSFL treatments with varying concentrations of BSFL (28%, 31% & 34%). The BSFL treatments were compared to the pork vienna sausage, with regard to proximate composition, texture profile analysis on day 1 and day 14 (refrigerated storage) and microbial safety (day 0 and day 14). The results showed that the pork treatment was higher ($p<0.001$) in protein and moisture than all of the BSFL treatments, however, there was no difference between treatments with regard to fat or ash content. Texture profile analysis indicated that the treatments had no effect on perceived hardness and cohesion on day 1. With regard to gumminess and springiness, the pork vienna sausage had the highest ($p<0.001$) gumminess values. After 14 days of refrigerated storage the pork treatment retained its hardness, however, there was a decrease ($p<0.001$) in the hardness of all of the BSFL treatments. Cohesion values differed between treatments on day 14 ($p<0.001$), but both the 34 % and 31 % treatment were comparable to the pork treatment. On day 1 there was a difference in springiness between the 34 % treatment and 28 % treatment ($p<0.05$), and only the control and 28 % treatment retained its springiness at day 14. Black soldier fly larvae sausages were considered to be microbiologically safe to eat at day 0 and after 14 days of storage under refrigerated conditions. This study established that the BSFL vienna-type sausages differ from pork viennas in terms of nutritional composition and perceived texture, however, out of the three BSFL treatments, the 28 % BSFL treatment compared the best to the pork treatment in terms of protein content, ash content, perceived hardness, cohesion and gumminess.

Keywords: Vienna sausage, black soldier fly larvae, meat alternative, processed meat

4.2 Introduction

With the world's population predicted to be 9.6 billion in 2050, there will be an increase in demand for food sources to feed everyone (Mitsuhashi, 2010; Premalatha *et al.,* 2011). It has been suggested that the demand for future food requirements will largely be in favour of protein based products, and meat production alone will need to increase to 200 million tons per annum by 2050 (Barnes, 2012). Western consumers are becoming aware of the environmental impact of consuming meat and are beginning to seek alternatives to animal protein in efforts to shift to a more sustainable diet (Schösler *et al.,* 2012). All of these factors have led to an increasing interest in insects as a protein source, yet consumer acceptance is one of the main barriers preventing the commercialisation of insects in the Western culture (van Huis *et al.,* 2013). In Western culture insects are not part of the cultural diet, resulting in a strong aversion towards incorporating insects into their diet (Hartmann *et al.,* 2015; Tan *et al.,* 2015). Investigations into whether Western consumers are ready to adopt insects as a meat alternative in their diet have yielded some conclusive and interesting results. In a study by Schösler *et al.* (2012), Western consumers were required to describe their willingness to try mealworms and locusts in various dishes. The consumers stated that they were willing to try insects in a dish, but were less likely to buy, consume or prepare meals where the insects were easily visible. Other studies yielded similar results, and it has been established that products with visible insects are met with more disgust and apprehension by Western consumers than those where insects are processed and disguised in the product (Hartmann *et al.,* 2015; Tan *et al.,* 2015; Caparros Megido *et al.,* 2016). The degree of processing also has an effect on consumer acceptance, and products where insects are completely disguised, such as insect flour, are more readily accepted than products were insects are still visible (Gmuer *et al.,* 2016). It has therefore been suggested that in order to introduce insects as a commercial food source into Western culture, insects should be incorporated into familiar food products that are currently consumed (Schösler *et al.,* 2012; Hartmann *et al.,* 2015; Tan *et al.,* 2015; Tan *et al.,* 2016). Further investigations have also established that the majority of the consumers associate insects with meat products and meat dishes, as opposed to other savoury or sweet options. Therefore, insects should be incorporated into a meat product in order to introduce insects into the Western food market (Caparros Megido *et al.,* 2016; Schouteten *et al.,* 2016).

Insects have been processed into burger patties (Caparros Megido *et al.,* 2016; Schouteten *et al.,* 2016), yet currently there is no research on the production of insects into cooked sausages. Vienna (hot dog) sausages are defined by their characteristic cylindrical shape with hemispherical ends and by the stable meat emulsion that is formed (Ranken, 2000). Emulsions are a two-phase colloidal system, consisting of two immiscible liquids, where

one liquid is dispersed as small particles in another liquid of a different composition, usually it comprises of an aqueous phase and a hydrocarbon phase (Allais, 2010). Vienna sausages are small calibre sausages made from meat and fat which is finely chopped into a homogenous batter, and filled into a casing. Thereafter it is smoked and cooked (Ranken, 2000; Allais, 2010). Vienna sausages, like most cooked sausages, are fully cooked to an internal temperature of 70 °C and then chilled rapidly to prevent microbial spoilage (Ranken, 2000).

It has been established that Western consumers would be more willing to consume insects in a processed form, specifically as a meat alternative. It is therefore the objective of this study to investigate whether black soldier fly larvae (BSFL) can be incorporated into a vienna-type sausage and be comparable to a traditional pork vienna sausage. Vienna sausages were chosen because they would easily disguise the BSFL when blended into the batter, whilst the traditional vienna processing will provide strong smoke and spice flavours to mimic traditional sausages and avoid disgust associated with potential unknown insect flavours. Typically in an emulsified meat sausage sodium chloride is added to extract the salt soluble myofibrillar proteins, namely actin and myosin, which allows the proteins to surround the fat molecules and bind water to form an emulsified batter. These salt soluble proteins in meat contribute to the characteristic structure found in emulsified sausages, which contributes to the texture and mouthfeel of the sausage (Allais, 2010). The concern when using BSFL, as with any alternative protein source in the vienna sausage, is the potential negative effect it would have on texture, due to the absence of meat proteins. Larvae of the black soldier fly were selected, as they are soft and they don't have a hard exoskeleton, and would therefore not influence the texture.

4.3 Materials and Methods

4.3.1 Experimental design

A randomised block design was used to compare the difference between a control vienna sausage (pork standard) and BSFL vienna sausages with three varying concentrations of BSFL (34 % BSFL, 31 % BSFL & 28 % BSFL). There were four different vienna sausage treatments produced, and five batches of each treatment.

5.3.2 Vienna sausage production

4.3.2.1 Ingredient preparation

All of the pork and pork fat was obtained from Winelands Pork (La Belle Rd, Stikland, Cape Town, South Africa), vacuum sealed and frozen at -20 °C until used. The BSFL was obtained from Agriprotein at 18 days old (Rochester Road, Philippi, Cape Town, South Africa), blanched for 3 min at 100 °C, vacuum packed and frozen at -20 °C until needed. The salt packs, vienna

spice packs, soya concentrate, carrageenan and collagen casings were obtained from Deli Spices (Bertie Avenue, Epping 2, Cape Town, South Africa) and the lecithin was obtained from CPJ Chemicals (Christian Ave, Epping 2, Cape Town, South Africa).

4.3.2.2 Production

At the time of this investigation there was no available information regarding BSFL as a meat alternative in vienna-type sausages, therefore pilot trials were conducted using a traditional vienna sausage recipe (obtained from Deli Spices) as a basis to start from. The details of the pilot trials are found in Annexure 1. The BSFL vienna formulation developed in the pilot trials was used as a basis for the BSFL vienna sausage treatments. Four different treatments were investigated to obtain a BSFL vienna sausage that was comparable to that of a commercial pork vienna sausage. Each batch consisted of 5 kg of ingredients, as this allowed for optimum functioning of the bowl chopper. The BSFL and soya concentration fluctuated inversely throughout the treatments. Lecithin and kappa carrageenan, were added only to the BSFL sausages to aid in binding, emulsification and water retention, as BSFL have weak emulsifying activities (Chapter 4). The formulations of each treatment are shown in Table 5.1.

Table 5.8 Formulation of treatments

Ingredient (%)	**Control**	**28 % BSFL**	**31 % BSFL**	**34 % BSFL**
Soya concentrate	3	9	6	3
Minced fat	19.56	19.56	19.56	19.56
Cold water	28.5	28.5	28.5	28.5
Potato starch	5	5	5	5
Spices	6.5	6.5	6.5	6.5
Salt pack	2.84	2.89	2.89	2.89
Pork	34.6	0	0	0
BSFL	0	28	31	34
Lecithin	0	0.5	0.5	0.5
Kappa Carrageenan	0	0.06	0.06	0.06
Total	**100**	**100**	**100**	**100**

Good manufacturing practises were strictly adhered to throughout the manufacturing process. All four treatments were produced in the same manner, with the exception of the

additional ingredients used in the production of the BSFL vienna sausages, namely lecithin and kappa carrageenan. Prior to production the pork, BSFL and fat was thawed at 4 °C for 12 h. A six blade bowl chopper (Manica CM-21, Equipamient os Carnicos, Spain) was used to process the sausages according to the flow diagram in Fig 5.1 until a sticky, shiny batter was formed. The temperature was carefully controlled, and the various ingredients were added into the bowl chopper at specific temperatures (Fig. 5.1). The final temperature of the batter in the bowl chopper was below the recommended 12 °C (Allais, 2010). A Talsa hydraulic sausage filler (DMD Foodtech, Epping, South Africa) was used to fill the sausage batter into the 25 mm collagen casings (Deli spices, Bertie Avenue, Epping 2, Cape Town, South Africa), which were then twisted every 20 cm to produce individual sausages. The sausage treatments were labelled, weighed and hung in a processing chamber (Reich Airmaster UKF 2000 BE, Urbach, Germany). The sausages were smoked and cooked to an internal temperature of 72 °C (Boles,2010), measured using a thermocouple probe that was inserted into the centre of a randomly selected sausage.

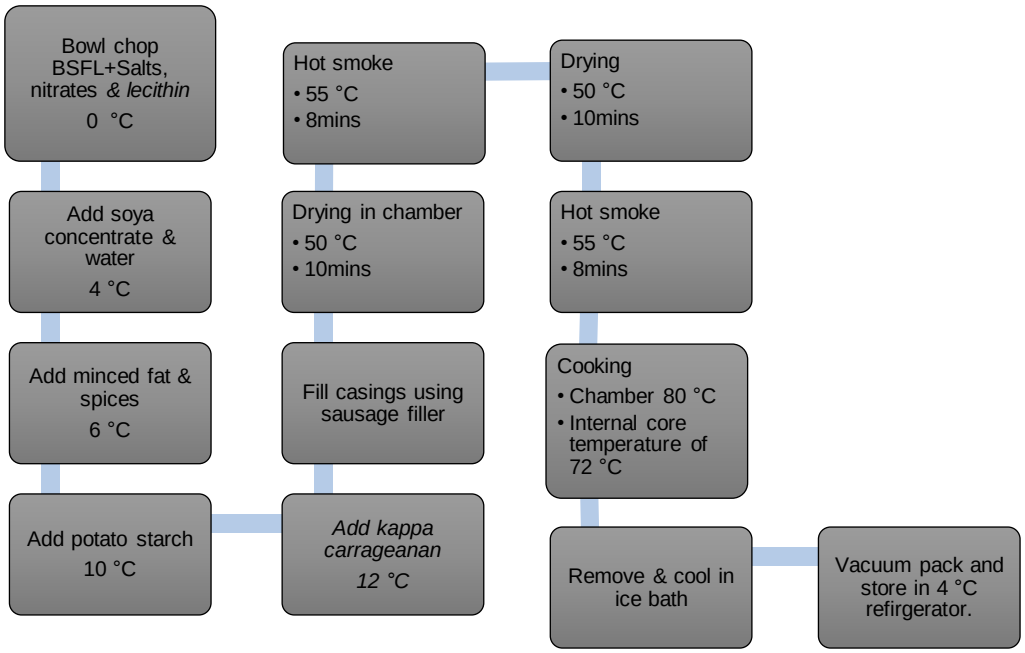

Figure 5.15 Flow diagram showing the sequence of steps in the production of the vienna sausages**,** where the words in italics are the ingredients only used in the black soldier fly vienna sausages, and were not included in the pork sausages.

4.3.3 Proximate analysis

Two sausages from each treatment and batch were tested for moisture content according to Official Method 934.01 (AOAC, 2002), ash content according to Official AOAC Method 942.05 (AOAC, 2002), crude fat using the 2:1 chloroform/methanol extraction method (Lee *et al.,* 1996) and protein content according to the Official AOAC Method 992.15 (AOAC, 1992) using the defatted samples from the chloroform/methanol extraction.

4.3.4 Physical analysis

The samples were cooled by submerging in ice water for 10 min, removed and dried. Percentage mass loss was calculated by weighing all the samples on a scale (Digi DS-673, Teraoka Seilko Co. Ltd, Japan) prior to placing the sausages in the chamber and immediately after removal from the chamber.

Texture profile analysis (TPA) was conducted using the method as described by Schutte (2008) with slight modification. TPA was conducted on day 1 and once again after 14 days of storage in a vacuum sealed bag at 4 °C to determine whether the sausage, using an Instron Universal Testing Machine (Instron 3345, Instron corp., MA, USA) and Bluehill 2 software (Instron Corporation, MA, USA). A 30 mm diameter circular plate attached to a 5 kN load cell was used to perform a cyclic compression test with a cross head speed of 100 $mm.min^{-1}$. Two sausages from each batch and treatment were selected, and five cores of 20 mm thick and 20 mm in diameter were cut from each sausage. The cores were placed on the platform, directly underneath and parallel to the anvil. The cyclic compression test was conducted with a 40 % compression to determine the hardness (N), gumminess ($N.cm^{-2}$), cohesion energy (ratio) and springiness (mm) of the sausages (Schutte, 2008). A 40 % compression was performed in order to obtain descriptive results, whilst still maintaining the integrity of the vienna structure.

4.3.5 Microbiological Analysis

Microbial analysis was conducted on the sausages treatments to determine the microbial activity on the vienna sausages and to determine whether the cooking steps were sufficient to ensure microbial safety of the sausages. Two randomly selected sausages from each batch and from each treatment were aseptically sampled. The samples from the batches within each treatment were pooled together, and tested in duplicate for total viable count (TVC), aerobic endospore formers, *Escherichia coli*, *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella.* Sausages were tested on day 0 for the initial microbial load post processing, and then again after 14 days of being vacuum sealed in refrigerated storage (4 °C), to give an indication of the shelf life of the vienna sausages.

For each test, 25 g of the pooled sausage samples were diluted in 225 ml of Buffered Peptone Water (BPW) in a stomacher bag, and then stomached for 2 min (Seward stomacher 400). A dilutions series (10^{-1} to 10^{-3}) of each pooled sample was prepared by adding 1 ml of each samples to 9 ml sterile Physiological Salt Solution (PSS). The dilution series was used to inoculate each agar plate using the spread plate technique. All agar and solutions were prepared according to manufacturer's specifications (Merck, South Africa). Total viable count was determined by spread plate on Tryptic Soy Agar (TSA) (Merck, South Africa), and then

incubated at 37 °C for 48 h (da Silva *et al.,* 2012; SANS, 2012). Aerobic endospores were tested by heating diluted sample to 75 °C for 20 min, and then spread plating it on TSA agar (Merck, South Africa) and incubating it at 35 °C for 48 h (Austin, 1998; Da Silva *et al.,* 2012). Enumeration of *E.coli* was tested by the spread plate technique on Violet Red Bile Glucose Agar (VRBGA) (Merck, South Africa) and incubated at 30 °C for 24 h (Da Silva *et al.,* 2012; Merck, 2007). Enumeration of *S. aureus* was done by means of the spread plate technique on Mannitol Salt Agar (MSA) (Merck, South Africa) which was incubated at 35 °C for 48 h (Gorwitz *et al.,* 2008).

The presence of *L. monocytogenes* was tested using a two-step procedure. Firstly, 25 g of each treatment was added to 225 ml of half strength Fraser broth and incubated at 35 °C for 24 h. Thereafter 0.1 ml of each incubated sample was transferred into 10 ml of full Fraser broth and incubated at 35 °C for 48 h. The half Fraser broth culture was then streaked onto Oxford agar plates (Merck, South Africa), and PALCAM agar plates (Merck, South Africa) and incubated micro-aerobically at 35 °C for 24 h (SANS, 2001). The same was performed with the culture from the full Fraser broth (SANS, 2001).

The presence of *Salmonella* was tested in four successive stages. Firstly 25 g of each treatment was added to BPW and incubated at 35 °C for 24 h (Da Silva *et al.,* 2012; SANS, 2003). Thereafter, 0.1 ml of the incubated samples were transferred to 10 ml of *Salmonella* enrichment broth (Merck, South Africa) and incubated for 42 °C for 24h, and then for a further 24 h at 35 °C (Da Silva *et al.,* 2012; SANS, 2003). The inoculated *Salmonella* enrichment broth samples were then streaked out on Xylose Lysine Deoxycholate (XLD) (Merck, South Africa) agar plates and incubated at 35 °C for 24 h (Da Silva *et al.,* 2012; SANS, 2003).

4.3.6 Statistical Analysis

Statistical analysis was conducted using Statistica version 12, Dell Inc. (2015). A complete randomised plot design with four treatments and five batches of each treatment were tested using a mixed model repeated measures ANOVA.

4.4 Results and discussion

5.4.1 Proximate analysis

Protein and moisture were significantly different between the treatments (Table 5.2), whereas there was only a slight difference between the ash and fat contents of the control sausage and the BSFL sausages. The control sausage had the highest moisture content ($P<0.001$). For the BSFL sausages, the 28 % BSFL sausage had the lowest moisture content and there were no differences between the 34 % and 31 % BSFL sausages. The low moisture content of the 28 % BSFL vienna was unexpected, as it contained the highest soya concentration, which is

known for its high water binding capacity, which was expected to retain more water during the cooking process (Joly & Anderstein, 2009). The moisture content of the 34 % BSFL and 31 % BSFL were similar (p=0.74), but they differed from both the control and 28 % BSFL (p<0.05). There were differences in protein content between each treatment. The control had the highest protein content (p<0.001), which was expected as BSFL typically has a slightly lower protein content (17.5 $g.100^{-1}g$ (wet basis)) (Finke, 2013) than pork (20–25 $g.100^{-1}g$ (wet basis)) (USDA, 2016). Within the BSFL treatments there was an increase (p<0.001) in protein content as the BSFL concentration decreased. This is attributed to the increase in soya concentrate, which has a higher protein content (63 $g.100g^{-1}$) in its powder form, than the BSFL. Although 28 % BSFL had the highest protein content out of the black soldier fly treatments, it was still substantially less (p<0.001) than the protein content of the control, therefore BSFL is not quite a desirable as a pork sausage in terms of its protein content. Up to date there is no research on insects in sausage products, therefore there are no findings to compare to the results found in this study.

Table 5.9 Means and standard deviations of proximate composition of the four vienna sausage treatments

	Treatments				
	Control	**34 % BSFL**	**31 % BSFL**	**28 % BSFL**	**LSD**
Moisture (%)	$58.2^{a} \pm 1.99$	$55.0^{b} \pm 1.18$	$55.3^{b} \pm 1.04$	$51.6^{c} \pm 0.91$	P<0.001
Ash (%)	$4.2^{b} \pm 0.36$	$4.7^{a} \pm 0.1$	$4.7^{a} \pm 0.17$	$4.5^{ab} \pm 0.51$	P=0.067
Fat (%)	$18.4^{b} \pm 2.15$	$21.0^{a} \pm 1.66$	$19.6^{ab} \pm 0.88$	$20.8^{a} \pm 1.13$	P=0.068
Protein (%)	$42.8^{a} \pm 0.93$	$34.4^{d} \pm 1.15$	$36.2^{c} \pm 1.03$	$39.2^{b} \pm 0.91$	P<0.001

*a.b.c.d Means within a row with different superscripts are significantly different (p <0.05); LSD- Least significant difference

4.4.2 Physical analysis

Many of the sausages burst and fell on the floor whilst cooking in the chamber, it was therefore not possible to calculate percentage mass loss during this process. There was a slightly higher number of the 34 % BSFL sausages that burst, however, it was inconsistent between batches and therefore no trend could be established. It is speculated that there was poor water binding in the BSFL sausages causing the free water to expand during heating, resulting in an increase in vapour pressure, causing the casings to burst.

Texture is primarily a sensory attribute determined best by the human senses, however, texture profile analysis (TPA) using the compression test gives a good indication of the perceived texture of products (Bourne, 1990). The texture profile of the sausages (Table

5.3) were defined by the following parameters: the maximum force (N), required to compress the sample, the cohesion energy was measured as a ratio of the total energy of the first compression and the total energy of the second compression indicating the extent to which the samples deformed during the compression, gumminess indicated the force (N) that would be required to disintegrate the sample for swallowing (hardness x cohesiveness), and springiness measured the samples ability to recover to its original form after compression (Mendoza *et al.,* 2001; Herrero *et al.,* 2008). The texture of food is derived largely from the structure of the food (Bourne, 1990) and due to the fact that there was no meat in the BSFL, it was expected that there would be differences in the texture profile between the control and the BSFL treatments. The results indicated the effects of treatment, time and treatment-time interaction on the texture of the sausages (Table 5.4). The vienna sausages were tested after 14 days of storage at 4 °C refrigeration to give an indication of the shelf-life quality of the product in terms of perceived texture and hardness (Table 5.3).

With regard to the hardness (max force) of the samples, there was an interaction ($p<0.001$) between treatment and time, however, there were no notable differences between the treatments with regard to perceived hardness on day 1 (Fig. 5.2). This result was unexpected, as the moisture content differed significantly between treatments (Table 5.2), and it was expected that the hardness would increase as the moisture content decreased (Li *et al.,* 1998; Pereira *et al.,* 2011). After 14 days of refrigerated storage the control retained its hardness, however, there was a drastic decrease ($p<0.001$) in the hardness of the BSFL treatments (Table 5.3; Fig. 5.2). Within the BSFL treatments on day 14, there was a notable increase in hardness (Fig. 5.2) which corresponded to the decrease in moisture content (Table 5.2). It is also known that soya isolate typically increases the hardness of emulsified sausages, and could have also contributed to the increase in hardness within the BSFL treatments (Ensor *et al.,* 1987; McCord *et al.,* 1998; Youssef & Barbut, 2011). These results indicate that there will be a noticeable change in perceived hardness in the BSFL sausages for consumers after 14 days of storage, therefore they are not expected to have a good shelf-life in terms of quality. At this stage it is unknown as to why the hardness of the BSFL decreased over 14 days, further investigations are required to explain this phenomenon and its correlation with the other parameters (such as water binding capacity).

Table 5.10 Means and standard deviation of texture analysis of the four vienna sausage treatments after refrigerated storage at day 1 and day 14

	Day 1				Day 14			
	Control	**34 % BSFL**	**31 % BSFL**	**28 % BSFL**	**Control**	**34 % BSFL**	**31 % BSFL**	**28 % BSFL**
Hardness (N)	$31.18^{a} \pm 5.91$	$31.62^{a} \pm 6.38$	$31.98^{a} \pm 4.46$	$33.43^{a} \pm 6.99$	$32.43^{d} \pm 8.37$	$4.19^{c} \pm 1.21$	$8.16^{bc} \pm 1.85$	$11.75^{b} \pm 1.69$
Gumminess (N)	$19.12^{b} \pm 6.61$	$2.47^{d} \pm 0.28$	$5.76^{d} \pm 2.15$	$9.12^{dc} \pm 0.53$	$31.42^{a} \pm 12.97$	$2.92^{d} \pm 0.85$	$6.52^{dc} \pm 0.90$	$13.13^{bc} \pm 1.47$
Cohesion Energy (A_1/A_2)	$0.63^{cd} \pm 0.09$	$0.36^{d} \pm 0.06$	$0.41^{d} \pm 0.14$	$0.35^{d} \pm 0.03$	$1.12^{ab} \pm 0.56$	$0.33^{d} \pm 0.14$	$0.89^{cb} \pm 0.20$	$1.28^{a} \pm 0.16$
Springiness (mm)	$6.74^{ab} \pm 2.02$	$8.14^{a} \pm 1.32$	$6.92^{ab} \pm 2.26$	$5.47^{cb} \pm 1.35$	$8.03^{a} \pm 1.15$	$3.17^{cd} \pm 0.42$	$4.25^{cd} \pm 0.31$	$5.52^{cb} \pm 0.36$

*a.b.c.d Means within a row with different superscripts are significantly different ($p < 0.05$).

Table 5.11 The P values for the main and interaction effects for texture profile analysis on the four sausage treatments on day 1 and day 14

	Hardness (N)	**Gumminess ($N.cm^{-2}$)**	**Cohesion Energy (A_1/A_2)**	**Springiness (mm)**
Treatment	0.000203	0.000003	0.000842	0.02411
Time	0.000325	0.057951	0.005264	0.02081
Treatment*Time	0.000114	0.087537	0.002072	0.00097

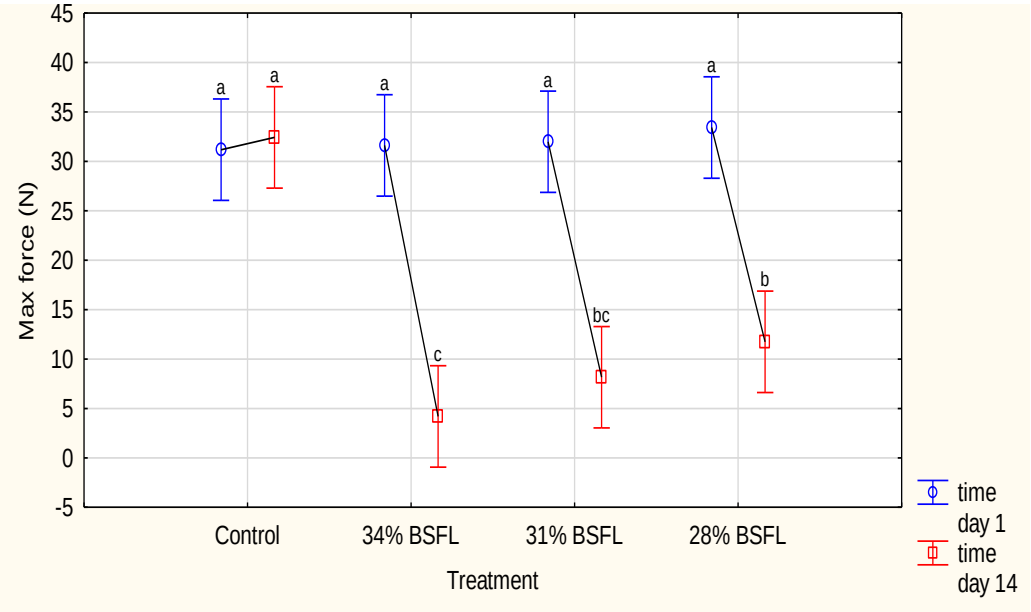

Figure 5.2 Hardness (mean max force values) of the four vienna sausage treatments for day 1 and day 14, where the means with different superscripts are considered significantly different.

There were no differences in gumminess values between day 1 and day 14 (Table 5.4; Fig. 5.3), however, there were differences ($p<0.001$) between the treatments. Overall, the control had the highest ($p<0.001$) gumminess values, which was expected, as soya has been found in previous studies to decrease the gumminess of meat products due to its dense structure (Ulu, 2004; Youssef & Barbut, 2011; Savadkoohi *et al.,* 2014). There were no differences in gumminess between the BSFL treatments on both day 1 and day 14 (Table 5.3; Fig. 5.3), indicating that the decrease in BSFL concentration did not affect the gumminess of the vienna sausage.

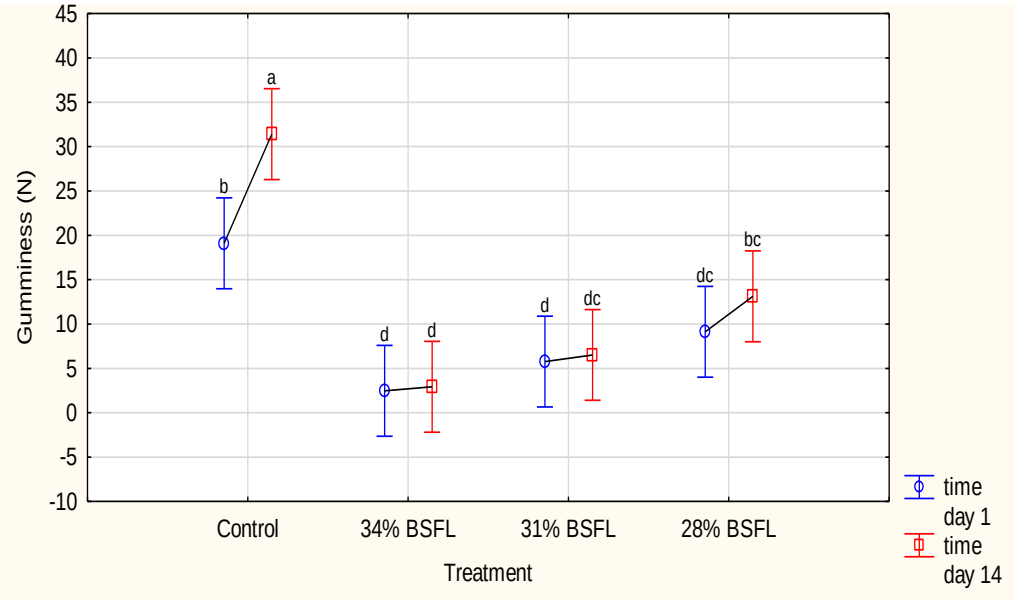

Figure 5.3 Mean gumminess values the four vienna sausage treatments for day 1 and day 14, where the means with different superscripts are considered significantly different.

On day 1, there were no differences in cohesion values between the treatments, however, on day 14 there were differences ($p<0.001$) between treatments (Table 5.3; Fig. 5.4). On day 14 the 34 % BSFL treatment had the lowest cohesion value and was the only treatment that differed ($p<0.001$) from the control treatment. The 34 % BSFL treatment was the only treatment that retained its cohesion values, whereas the cohesion values of the other treatments all increased ($p<0.001$) between day 1 and day 14.

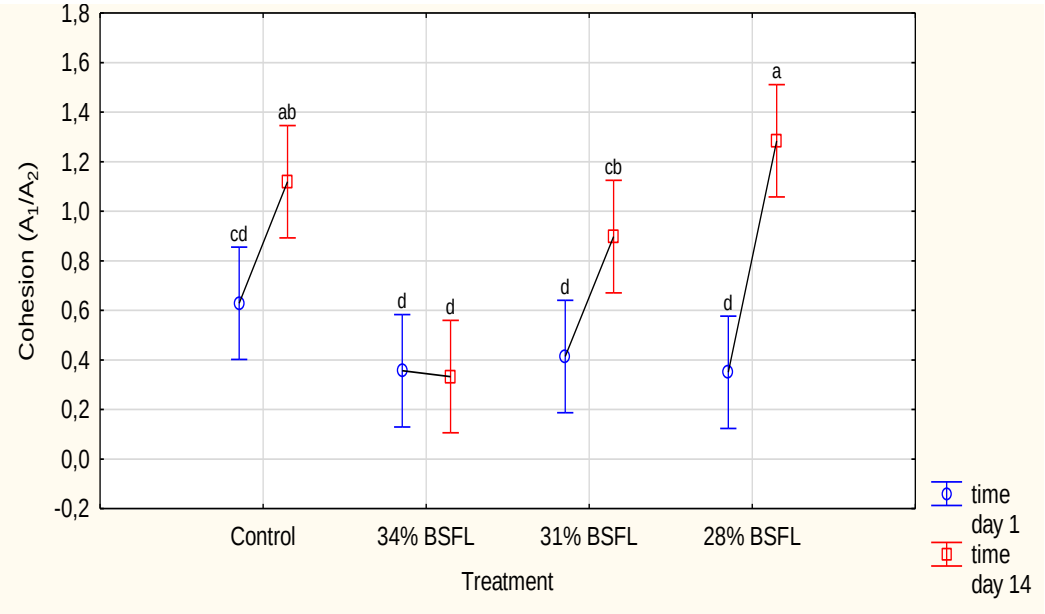

Figure 5.4 Mean cohesion values the four vienna sausage treatments for day 1 and day 14, where the means with different superscripts are considered significantly different.

There was an interaction ($p<0.001$) between treatment and time with regard to the springiness of the vienna sausages (Table 5.4). On day 1 the BSFL treatments compared well to the control treatment, with little variation in the springiness between the treatments, with the exception of the 28% BSFL treatment which was significantly lower. The only difference between treatments on day 1 was between the 34 % BSFL and 28 % BSFL treatment ($p<0.05$). The control retained its springiness at day 14, as did 28 % BSFL, but the springiness of the 34 % BSFL and 31 % BSFL treatments decreased substantially ($p<0.001$) over 14 days (Table 5.3; Fig. 5.5). There was also a slight increasing trend within the BSFL treatments on day 14 where the springiness increased as the BSFL concentration decreased. This increase in springiness could be attributed to the increase in soya concentration/decrease in moisture content, both of which have been found to increase springiness of sausages (Li *et al.,* 1998). Overall, the BSFL treatments did not compare favourably to the control with regard to springiness on day 14 and the results from this study are consistent with previous findings that non-meat ingredients have been found to decrease the springiness of meat sausages (Li *et al.,* 1998; Ulu, 2004; Youssef & Barbut, 2011).

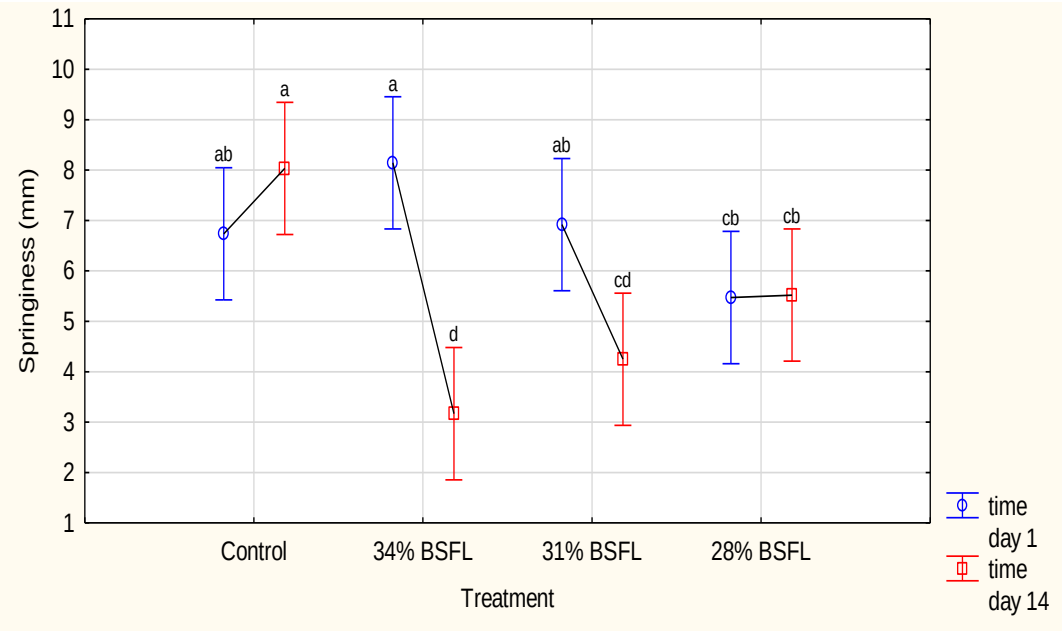

Figure 5.5 Mean springiness values the four vienna sausage treatments for day 1 and day 14, where the means with different superscripts are considered significantly different.

Overall, it is evident from the results that there is a reaction that occurs in the BSLF vienna sausages over time, causing significant changes in the textural properties. It could be as a result of a weak protein structure within the BSFL vienna sausages or protein denaturation. The exact cause is unknown at this stage, however, it is something that will need to be investigated prior to commercialisation of BSFL vienna sausages.

4.4.3 Microbiology

Microbial analysis was conducted post processing on the sausage treatments, in order to ascertain whether the processing parameters were adequate enough to ensure that the sausages were safe for human consumption, as well as, to determine which micro-organisms could potentially be associated in the production of BSFL vienna sausages. There are no regulations for insects, and since the BSFL were processed into vienna sausages and contained both pork fat and high protein content, the guidelines and legislation for microbial limits in processed meat products were considered in this investigation.

Total viable count (TVC) and aerobic endospores were tested to determine the general quality of the product and to give an indication of the general hygiene and potential shelf-life (Jay *et al.,* 2005). All of the sausage treatments were within specifications with regard to total viable count, indicating good hygiene during production (Table 5.5) (DOH, 2014). The main aerobic endospore former of concern in cooked meats is *Bacillus cereus. Bacillus cereus* produces enterotoxins that are heat tolerant, and can withstand the thermal treatments in sausage production. Regulations for processed meat stipulate that there should be <100 cfu.g^{-1} of aerobic endospore forming bacteria (DOH, 2014).

Table 5.5 Results of microbiological analysis on the four vienna sausage treatments at day 0 and after 14 days of refrigeration reported in cfu.g^{-1}

	Treatments								
		Day 0				Day 14			
	Guidelines	**Control**	**34 % BSFL**	**31 % BSFL**	**28 % BSFL**	**Control**	**34 % BSFL**	**31 % BSFL**	**28 % BSFL**
Total viable count	<2x10^5	5000	500	100	10	1200	350	900	100
Aerobic endospores	<100	1200	50	10	10	100	ND	ND	10
E.coli	<10	ND	ND	ND	ND	ND	ND	ND	ND
S.aureus	<100	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella spp.	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g	ND in 25g
Listeria spp.	<10	ND	ND	ND	ND	ND	ND	ND	ND

ND-None detected

Department of Health. (1972). Foodstuffs, cosmetics and disinfectants Act, 1972 (Act No. 54 of 1972), Regulations governing microbiological standards for foodstuffs and related matters (R.692 of 1997). [Internet Document].09/03/2015.

With regard to aerobic endospore formers, all the BSFL sausages were regarded as safe to consume, however, there was more growth found on the control vienna sausage and they exceeded the specifications stipulated by the Department of Health (DOH). This could be as a result of contamination in the pork meat. Indicator organisms, namely *E.coli* and *S.aureus,* as well as, pathogens commonly associated with chilled processed meat products, namely *Salmonella* and *Listeria monocytogenes* (Boles, 2010; Yu *et al.,* 2016) were also investigated. The results show that there was no *E. coli, Salmonella*, *S. aureus* or *Listeria* detected in any of the vienna sausage treatments. This was expected as *E. coli, S.aureus, Salmonella* and *Listeria* are killed at temperatures above 70 °C (Nørrung *et al.,* 2009; Boles, 2010), and the vienna sausages were cooked to a core temperature of 72 °C. Indicator organisms are helpful in assessing potential spoilage or safety hazards that could occur on the food product, as a result of poor hygiene standards or contamination throughout production (Baylis *et al.,* 2011). The fact that there were no indicator or common pathogenic organisms present in any of the sausage treatments is a good indication that the hygiene standards and the cooking step were sufficient to ensure microbial safety of the product (Baylis *et al.,* 2011). According to the specifications set out by the DOH, the BSFL sausages are considered safe for human consumption on both day 0 and day 14 (DOH, 2014).

Vienna sausages are perishable and are required to be stored at refrigerated temperatures. *L. monocytogenes* is a concern in chilled, ready to eat products, as it can survive and proliferate in refrigerated conditions. *Listeria* contamination occurs post cooking, through unhygienic handling and packaging (Nørrung *et al.,* 2009). On day 14 there was no *E. coli, Salmonella*, *S. aureus* or *Listeria* detected in any of the vienna sausage treatments, and both the TVC and the aerobic endospore formers were within specifications for human consumption (DOH, 2014). These results indicate correct cooking temperatures and good post production hygiene. The cooking step not only reduced the initial microbial load to satisfactory levels, but it also played a significant role in extending the shelf-life of the vienna sausages (Boles, 2010). In addition, wood smoking is known to produce antimicrobial compounds (Toldrá, 2010), which aids in extending shelf-life and along with the addition of salts and nitrates these hurdles contributed to the shelf-life of the vienna treatments (Simpson & Sofos, 2009). According to the specifications set out by the DOH, the BSFL sausages and the pork sausages are considered safe for human consumption (DOH, 2014).

4.5 Conclusions

The focus of this study was to determine whether BSFL could successfully be processed into a vienna-type sausage that was comparable to a traditional pork vienna. With regard to the proximate analysis, there were only differences between the treatments with regard to protein

and moisture content, where the control had the highest moisture and protein content. Within the BSFL treatments, the moisture content decreased as the BSFL concentration decreased, and oppositely the protein content increased as the BSFL decreased. Out of the three BSFL treatments, the protein content of 28 % BSFL compared best to the control, whilst the moisture content of 34 % BSFL compared best to the control.

The TPA indicated no notable differences between the treatments on day 1 with regard to perceived hardness and cohesion. With regard to the springiness on day 1 there was only a difference ($p<0.05$) between 34 % BSFL and 28 % BSFL treatment. The control had the highest gumminess values ($p<0.001$), and gumminess of the BSFL treatments were all similar. In the BSFL treatments the decrease in BSFL resulted in an increased cohesion and springiness, however, it had no effect on the gumminess of the BSFL treatments. On day 14 there was a drastic decrease ($p<0.001$) in the hardness of the BSFL treatments and the springiness of 34 % BSFL and 31 % BSFL. Inversely, on day 14 there was an increase in cohesion in the control, 31 % BSFL & 28 % BSFL viennas. These results indicate that there will be a noticeable change in perceived texture for consumers after 14 days of storage, and therefore BSFL vienna sausages may not have a good shelf-life in terms of quality. From a food safety standpoint, the results of the microbial analysis provided useful information pertaining to the safety of processing BSFL. According to regulations stipulated by the Department of Health (2014), BSFL sausages are considered safe to eat at day 0 and after 14 days vacuum sealed in refrigerated conditions.

Overall, it was established that the BSFL sausages do not behave like pork sausages, however, of the three BSFL treatments, the 28 % BSFL sausage compared best to the control in terms of protein content, ash content, perceived hardness, cohesion and gumminess. It would therefore be recommended that the 28 % BSFL formulation be used for further investigations such as sensory evaluation for taste and to compliment the TPA results. The 28 % BSFL formulation could also be used as a basis for further processing investigations to further improve texture and water binding capacity, which will improve the chances of acceptability by consumers.

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Chapter 6

General conclusions and recommendations

This study comprised of three investigations. Firstly, the proximate, chemical and microbial analysis of five different insect species; namely *Tenebrio molitor* (mealworm) larvae, *Blatta lateralis* (Turkistan roach), *Blaptica dubia* (orange spot roach), *Hermetia illucens* (black soldier fly) larvae and *Naupheta cinerea* (lobster cockroach) was conducted in order to determine the nutritional significance of consuming insects. For the second investigation, the black soldier fly larvae (BSFL) were selected for further analysis on its functional properties as they are considered by the European Union to have the biggest potential to be used in food. Lastly, the BSFL were used as a meat alternative in the production of a vienna-type sausage, whilst comparing them to traditional pork vienna sausages in terms of its nutritional composition, perceived texture analysis and microbial safety.

The results reiterated that insects are a good source of nutrients and can substantially contribute to one's daily nutritional requirements. *Tenebrio molitor* was found to have the highest fat content (35.7 g.100g^{-1}), and *B. lateralis* was found to have the highest protein content (101.5 g.100g^{-1}). The insects were also considered to be a good source of energy (averaging 24.1 MJ.kg^{-1}), crude fibre (8.7 g.100g^{-1} in *T. molitor* to 19.1 g.100g^{-1} in *H. illucens)* and minerals, specifically iron and zinc. The amino acid profile of each insect species compared favourably to the daily requirement for the average adult, with the exception of methionine, which is considered to be the limiting amino acid in all of the insects tested. These insects would therefore be best consumed in conjunction with foods that are high in methionine. Oleic acid was the most prominent fatty acid in all of the insects tested, with values ranging from 11.6 % in blanched *H. illucens* to 46.2 % in *B. dubia*. Linoleic acid was the highest polyunsaturated fatty acid and ranged from 3.3 % in blanched *H. illucens* to 13.9 % in blanched *B. lateralis*. The only omega-3 fatty acid, α-linolenic acid, was found in low concentrations in the insect species. The insects all contained relatively high saturated fatty acid concentrations with palmitic acid and stearic acid being the most abundant saturated fats. This could negatively affect the marketability of the insects, as consumers are becoming increasingly aware of the negative impact of consuming large amounts of saturated fats. In terms of microbial safety, *T. molitor* and *H. illucens* contained high TVC and high levels of *Enerobacteriaceae.* Blanching reduced the microbial levels to below the recommended amount (<10 cfu.g^{-1}). The aerobic endospore count was low on both *T. molitor* (<10 cfu.g^{-1}) and *H. illucens* (<100 cfu.g^{-1}). Salmonella was not found on either insect, however, *Listeria* species could be a potential problem, as there was slight colony growth. Overall, blanching is

suggested prior to eating or further processing insects, as it significantly reduces all microbial levels to safe levels for human consumption.

The results from the second investigation indicated that BSFL do have some functional properties, however, the extent of the functionality of BSFL in its whole form is somewhat limited. BSFL can be used to aid in water and lipid absorption, gelling and antioxidant activity, however, they do not function as well as dried insects. Equally, BSFL do not have strong emulsifying activities, and would need additional ingredients to aid in emulsification. Blanching showed inconsistent results by reducing some of the functional properties, as well as, having no effect on other functional properties such as water and lipid absorption capacity. Blanching prevented discolouration of the BSFL, and was seen to reduce microbial load (Chapter 3). Considerations would therefore need to be made regarding the final product and functional properties needed from the BSFL before deciding to use blanching as pre-processing treatment.

The final investigation focused on whether BSFL could successfully be processed into a vienna-type sausage that could be comparable to a traditional pork vienna. The BSFL were successfully processed into sausages, however, they were found to be different to the pork vienna sausage in terms of moisture, protein content, hardness (day 14), gumminess and springiness. It was determined that the 28 % BSFL sausage compared best to the control pork vienna sausage in terms of protein content, hardness, cohesion and springiness. From a food safety standpoint, the BSFL sausages are considered safe to eat at day 0 and after 14 days vacuum sealed in refrigerated conditions. The results provide useful information pertaining to the safety of processing BSFL.

Ultimately, this study has established that insects, and more specifically BSFL, have the potential to be a good alternative to meat in terms of its nutritional composition and processing potential. Insects as food is a new branch of scientific research with many avenues to pursue, therefore the recommendations for future research is endless. With regard to the functional properties, a more in depth study into ways to manipulate the functional properties of BSFL could lead to more efficient ways of processing it. Further research could include various processing conditions such as drying at various pH and ionic concentrations that optimise their functional properties. Research into the functional properties of other insect species could also yield different results, and could provide crucial information about a variety of insects with the potential for different applications.

With regard to the production of this BSFL sausage, further research into methods to prevent the textural degradation over time would need to be conducted. Possible solutions could be the addition of various water binders and texture enhancers to aid in stabilising the

structure of it. It would also be worth investigating the use of dried BSFL in the production process, as the water inclusion would be easier to control and manipulate throughout. With the high unsaturated fatty acid content, oxidative rancidity could be of concern for the BSFL sausages, and would also need to be investigated. Various antioxidants could be investigated to prevent oxidation in the sausages over an extended shelf-life. Another option could be investigating the potential of BSFL in other food products, specifically in its dried form. Insect powders could be used in a variety of products, and could perform many different functions, such as fortifying agents in baked goods, extenders in meat products and nutrient supplements in protein and snack bars. Processing techniques, such as extrusion, could also be employed to manipulate the texture of the insects in order to create new and enjoyable products.

In terms of sensory sciences, there is now a vast field with new and interesting sensory attributes to discover and profile. To date, there is only cultural knowledge on the sensory attributes of many insect species, however, there have been no efforts to scientifically investigate and document these. Furthermore, sensory evaluation of the BSFL sausages would also be able to determine whether there is a potential for them in the Western food market, as well as, aiding in defining the sensory qualities expected from insect products. Essentially, a new door has opened in the field of food science and the possibilities are endless.

Annexure 1

Pilot trials for formulation development and processing optimisation.

There was no available information regarding using larvae as a meat alternative in vienna sausages, therefore pilot trials were conducted using a traditional hot dog sausage recipe as a basis to start from. The base recipe was tried and tested and altered slightly during each step in the pilot trial to develop the optimum formulation. Each batch worked on a total of 5 kg, as this was the weight which ensured the optimum functioning of the bowl chopper. Table 1 shows the formulations that were used and developed throughout the pilot trial process.

Table 1 Vienna sausage formulation throughout pilot trials

Ingredient	**Pilot trial 1**	**Pilot trial 2**	**Pilot trial 3**
Soya concentrate	3 %	5 %	6 %
Minced fat	20 %	19 %	20 %
Ice	33 %	33 %	29 %
Potato starch	3 %	3 %	5 %
Spices	6.66 %	6 %	6 %
Salt	2.5 %	2.5 %	2.89 %
BSF Larvae	32.3 %	32 % (unblanched)	31 %
Lecithin	/	/	0.5 %
Kappa Carrageanan	/	/	0.15 %
Total	**100 %**	**100 %**	**100 %**

In pilot trial 1 and 2 the production process was the same as that of traditional hot dog sausage process, however, it was altered for the third pilot trial to accommodate the additional ingredients that are not usually associated with producing pork sausages. These processing steps are seen in the flow diagrams in Figures 1 and 2.

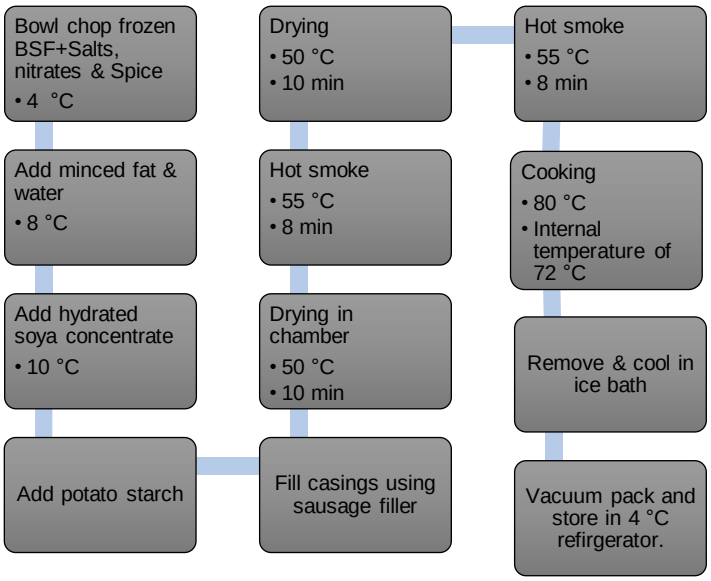

Figure 16 Flow diagram of the vienna sausage making process in pilot trial 1 and 2.

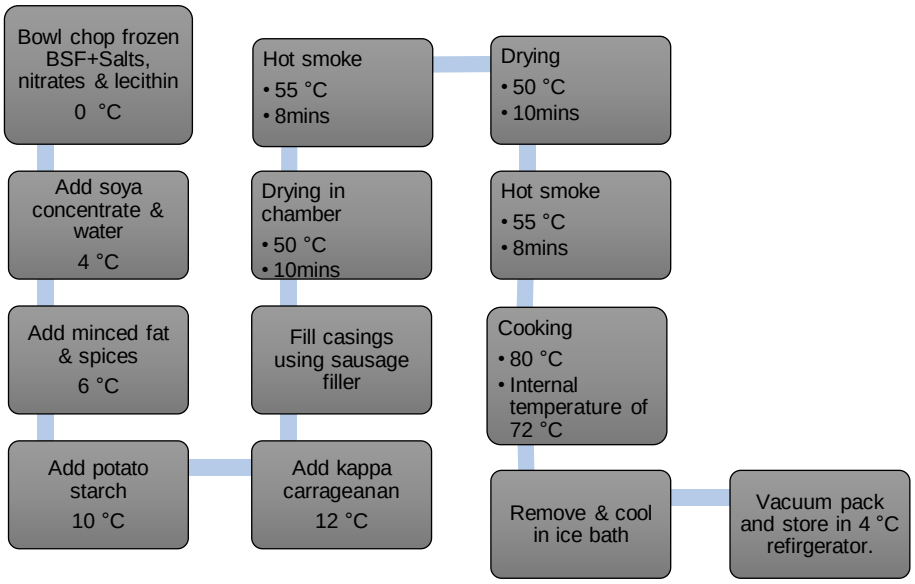

Figure 2 Flow diagram of the vienna sausage making process in pilot trial 3.

Pilot trials

During the production of pilot trial 1 there was a definite point where the consistency of mixture in the bowl chopper changed and lost its adhesiveness, this was as a result of over chopping the mixture. Additionally, at this point the mixture appeared to lose its shiny appearance and there was visible water separation, indicating poor emulsification. This gave an indication that the BSFL did not have strong enough emulsification properties to hold the pork fat and water together. It was speculated that the lack of binding and emulsification properties in the batter could have been as a result of the blanching of the insects, which could have denatured the

functional proteins. The resulting product was runny and did not have the desirable firm texture of a vienna sausage. The vienna sausages, oozed from the casings when cut and had a grainy mouth feel.

In pilot trial 2, the batter in the bowl chopper resembled the consistency of porridge, instead of that of sticky putty which is expected for an emulsified sausage. However, it retained water better than in the first pilot trial, and it stuck together well with no water separation, this was as a result of the additional soya added, and potentially the fact that the BSFL was not blanched prior to processing. The resulting products were not all consistent, as some of the casings split, and the casings were wrinkled to various degrees. The split casings could have been caused by the high unsaturated fat content of the BSF larvae. Additionally, there was noticeable water loss, as indicated by the wrinkled casings, indicating poor water binding in the mixture. The appearance was not appetising, as the colour was an off-putting dark brown colour and the casings were wrinkled. The dark colour was as a result of not blanching the BSF larvae prior to processing. There was a noticeable difference in colour of the mixture in the sausage filler from the top layer of the mixture which was exposed to oxygen and went black and the bottom layer which was not exposed to oxygen and remained beige. This can clearly be seen in Figure 2. The taste was consistent with that of traditional pork sausages, and it had a desirable, meaty texture. It was slightly dry, as a result of the excessive water loss. There was a slight after taste that resembled the after taste of corn starch flour. Overall, the mouth feel was a huge improvement from that of the first pilot trial.

Figure 3 The batter in the sausage filler, demonstrating the stark colour difference.

In pilot trial 3 the batter in the bowl chopper retained water and maintained the consistency of porridge as seen in pilot trial 2, yet it had a sheen which is expected for an emulsified mixture. The resulting products were all consistent, there was no water separation and none of the casings were wrinkled or split, indicating better water binding in the mixture which could be as a result of the combination of the additional soya, and the addition of the

lecithin and carrageenan. The taste was consistent with that of traditional smoked pork sausages, and it had a desirable, meaty texture. It was moist, as there was no water loss observed as in the previous two pilot trials. The colour was a light brown, resembling a soya Vienna, but it was an improvement on pilot trial 2 where the sausages were a dark brown. This indicated that the blanching of the insects is essential in pre-processing as it aids in colour retention. Overall, the formulation was a huge improvement from the first two pilot trials, and resembled a traditional pork Vienna in all regards except for the colour.

Conclusion

The formulation and processing parameters in pilot trial 3 was selected as the optimum formulation and process in order to produce vienna sausages using BSFL as the meat.